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Les documents fixés à cette attestation sont conformes à la version initialement déposée de la demande de brevet européen spécifiée à la page suivante.

Patentanmeldung Nr.

Patent application No. Demande de brevet n°

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PRIORITY DOCUMENT

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Bezeichnung der Erfindung/Title of the invention/Titre de l'invention: (Falls die Bezeichnung der Erfindung nicht angegeben ist, siehe Beschreibung. If no title is shown please refer to the description. Si aucun titre n'est indiqué se referer à la description.)

Method of producing double low restorer lines of brassica napus having a good agronomic value

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The invention relates to a method of producing a double low restorer lines of Brassica napus for Ogura cytoplasmic male sterility (cms) presenting a radish introgression carrying the Rfo restorer genes deleted of the radish Pgi-2 allele and recombined with the Pgi-2 gene from Brassica oleracea, and having a good agronomic value characterized by female fertility, a good transmission rate of Rfo and a high vegetative vigour. The invention relates also to a method of forming Brassica napus hybrid seed and progeny thereof and to the use of markers for selection.

Breeding restorer lines for the Ogu-INRA Cytoplasmic Male Sterility (cms) system in rapeseed (Brassica napus L.) has been a major objective during the past few years. Extensive backcross and pedigree breeding were necessary to improve their female fertility and to get double low restorer lines. The so-called « double low » varieties are those low in erucic acid in the oil and low in glucosinolates in the solid meal remaining after oil extraction. However some difficulties can still be encountered in breeding these lines (introgression rearrangements, possible linkage with negative traits) due to the large size of the radish introgression.

The inventors thus assigned themselves the objective of providing a new improved double low restorer line with a good agronomic value.

This objective is obtained by a new method of producing a recombined double low restorer line for the Ogu-INRA cms in rapeseed.

A first object of the present invention relates to a method of producing double low restorer lines of Brassica napus for Ogura cytoplasmic male sterility (cms) presenting radis introgression carrying the Rfo restorer gene deleted of the radish Pgi-2 allele and recombined with the Pgi-2 gene from Brassica oleracea, and having a good agronomic value characterised by female fertility, a good transmission rate of Rfo and a high vegetative vigour, said method including the step of:

- a) crossing double low cms lines of spring Brassica napus comprising a deleted radish insertion with the double low line of spring Drakkar for forming heterozygous restored plants of Brassica napus,
- 30 b) irradiating before meiosis the heterozygous restored plants obtained in step a) with gamma ray irradiation,

- c) crossing pollen from flowers obtained in step b) with the cms double low spring Wesroona line,
- d) testing the progeny for vigour, female fertility and transmission rate of the cms gene,
- 5 e) selecting progeny lines.

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A method according to claim 1, wherein the irradiation dose in step b) is 65 Gray during 6 mn.

According to one advantageous form of embodiment of the method according to the present invention, the double low cms line of spring Brassica napus of step a) is R211.

R211 is an INRA spring restorer line.

Drakkar is a French spring registered variety.

Wesroona is an Australian spring registered variety.

According to one advantageous form of embodiment of the method according to the present invention, the testing is performed with the combination of five markers selected from PGIol, PGIUNT, PGIint, BolJon and CP418.

Another object of the present invention relates to double low restorer lines of Brassica napus for Ogura cms presenting a Rfo insertion deleted of the radish Pgi-2 allele and recombined with the Pgi-2 gene from Brassica oleracea, and having a good agronomic value characterised by female fertility, a good transmission rate of

20 good agronomic value characterised by female fertility, a good transmission rate of Rfo and a high vegetative vigour.

According to one advantageous form of embodiment, the double low restorer lines present a unique combination of five markers selected from PGIol, PGIUNT, PGIint, BolJon and CP418.

- Another object of the present invention relates to a method of forming Brassica napus hybrid plants and progeny thereof obtained though the steps of:
  - a) providing a restorer line produced according to claim 1 and bred to be homozygous,
  - b) using said restorer line in a hybrid production field as the pollinator,
- 30 c) using cms sterile plants in a hybrid production field as the hybrid seed producing plant, and
  - d) harvesting the hybrid seed from the male sterile plant.

Another object of the present invention relates to seeds of Brassica plant obtained from the methods according to the present invention.

Still another object of the invention relates to seeds of Brassica napus deposited in NCIMB Limited, 23 St Machar Drive, Aberdeen, Scotland, AB24 3RY, UK, on July 4, 2003, under the reference number NCIMB41183.

Another object of the present invention relates to the use of at least four markers PGIol, PGIint, BolJon and CP418, or any portion of them comprising at least one polymorphic site, for characterising recombined restorer lines of Brassica napus for Ogura cms presenting a Rfo insertion deleted of the radish Pgi-2 allele and recombined with the Pgi-2 gene from Brassica oleracea, and having a good agronomic value characterised by female fertility, a good transmission rate of Rfo and a high vegetative vigour.

In a preferred embodiment, the combination is of five markers PGIol, PGIUNT, PGIint, BolJon and CP418.

- In the present invention, the expression " any portion of them comprising at least one polymorphic site" means any part of the sequence showing at least a difference between the B.oleracea type sequence and B.rapa type sequence.
  - Such markers are represented in the following figures and sequence listing for the R2000 line.
- 20 According to one advantageous form of embodiment, the present invention relates to:
  - The marker PGIol which is amplified using the primers: PGIol U and PGIol L
     (PGIol U: 5'TCATTTGATTGTTGCGCCTG3';
     PGIol L: 5'TGTACATCAGACCCGGTAGAAAA3')
- The marker PGIint which is amplified using the primers: PGIint U and PGIint L
   (PGIint U: 5'CAGCACTAATCTTGCGGTATG3';
   PGIint L: 5'CAATAACCCTAAAAGCACCTG3')
  - The marker PGIUNT which is amplified using the primers: PGIol U and PGIint L: (PGIol U: 5'TCATTTGATTGTTGCGCCTG3';
- 30 PGIint L: 5'CAATAACCCTAAAAGCACCTG3')

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- The marker BolJon which is amplified using the primers: BolJon U and BolJon L: (BolJon U: 5'GATCCGATTCTTCTCCTGTTG3';

BolJon L: 5'GCCTACTCCTCAAATCACTCT3')

- The marker CP418 which is amplified using the primers: SG129 U and pCP418 L:

(SG129 U: cf Giancola et al, 2003 Theor Appl. Genet. (in press)

pCP418 L: 5'AATTTCTCCATCACAAGGACC3')

5 Another object of the present invention relates to the PGIol, PGIUNT, PGIint, BolJon and CP418 markers whose sequences follow:

# PGIol R2000 marker:

	TCATTTGATT	GTTGCGCCTG	TCGCCTTGTT	GTGTTATGAT	GAATGAACAG	CAGTCATTTA	60
	ACATGTGGTT	AACTTAACAG	GGCTCCGGCT	GTTGCAAAAC	ACATGGTTGC	TGTCAGCACT	120
10	AATCTTCCGG	TATGAATTTG	TGATTAAATT	TGTTTGTTTG	TGACTCTTTC	TTCATTGTTC	180
i,	CTTTTTCTTCCTAC	AATAAACCGA	ATGTATAATC	TTTTTACAAA	CTGAATTTTC	TACCGGGTCT	240
	CATCUACA	1211121100011					248

### PGIUNT R2000 marker:

15	TCATTTGATT	GTTGCGCCTG	TCGCCTTGTT	GTGTTATGAT	GAATGAACAG	CAGTCATTTA	60
10	ACATGTGGTT	AACTTAACAG	GGCTCCGGCT	GTTGCAAAAC	ACATGGTTGC	TGTCAGCACT	120
	AATCTTGCGG	TATGAATTTG	TGATTAAATT	TGTTTGTTTG	TGAÇTCTTTC	TTCATTGTTC	180
	GTTTTCGTAC	AATAAACCGA	ATGTATAATC	TTTTACAAAC	TGAATTTTCT	ACCGGGTCTG	240
	ATGTACAATG	CTAGTCTCCA	TGTTCTTGGG	GATCATGATT	TATTTTCTAC	ATGTATTCAG	300
20	ACAGTACAGA	AGAAAGTGTT	CAAAACTCTG	GATGTTTTAA	TTTACAGTTA	GTGGAGAAGT	360
	TCGGCATTGA	TCCGAACAAT	GCATTTGCAT	TTTGGGACTG	GGTTGGTGGA	AGGTACAGTG	420
	GTAAGTGCTT	GTTTATTTGG	TTGTATAAAT	TTCTCGTCCA	TTTCCGCTTG	CTTAGTGTAT	480
	AACTGAAATT	CTTTTGCAGT	TTGCAGTGCT	GTTGGAGTCT	TACCATTGTC	TCTACAGTAT	540
	GGCTTCTCTG	TGGTTGAGAA	GTACGGTACC	TTCTACTTTA	TCAGCCATCT	CATAAAATGT	600
25	CTTAGGCATA	TTCTTTCTAT	TTTATTTCCC	TCTTAATGAT	TTCTTCTTTT	TTTTATTGCA	660
	TTCCCGTTTT	ATTTTCAAAA	GTTGTTACTG	TCTCTAAATC	AAGAAGAAAC	CTTCTTAGTA	720
	GATCCAGCTG	ATATTCAGCC	TTTTTTAAAT	TGGACTGCAG	GTTTTTAAAG	GGGAGCTTCA	780
	AGCATTGATA	AGCATTTCCA	GTCCACACCG	TTTGAGAAGA	ATATACCCGT	GAGTTGCATT	840
	AGTTGTGTGA	TTATACAGTT	TTCTTGTCTT	TTTGCTATGT	CCATCAACAC	TAGAGATTCG	900
30	TGAAGTTATT	AGTGTAGTCA	ACGCATAGGG	AGAGGTGATT	GGTGACTTTT	GGACGATTTC	960
	AGGTGCTTTA	GGGTTATTG					979

# PGIint R2000 marker:

	CAGCACTAAT	CTTGCGGTAT	GAATTTGTGA	TTAAATTTGT	TTGTTTGTGA	CTCTTTCTTC	60
35	ATTGTTCGTT	TTCGTACAAT	AAACCGAATG	TATAATCTTT	TACAAACTGA	ATTTTCTACC	120
	GGGTCTGATG	TACAATGCTA	GTCTCCATGT	TCTTGGGGAT	CATGATTTAT	TTTCTACATG	180
			AAGTGTTCAA	AACTCTGGAT	GTTTTAATTT	ACAGTTAGTG	240
			GAACAATGCA	TTTGCATTTT	GGGACTGGGT	TGGTGGAAGG	300
	00				TCGTCCATTT		360
	TACAGTGGTA	AGTGCTTGTT	TATTTGGTTG	TATAAATTTC		CCGCTTCCTT	
40	AGTGTATAAC	TGAAATTCTT	TTGCAGTTTG	CAGTGCTGTT	GGAGTCTTAC	CATTGTCTCT	420
10	ACAGTATGGC		TTGAGAAGTA	CGGTACCTTC	TACTTTATCA	GCCATCTCAT	480
			TTTCTATTTT	ATTTCCCTCT	TAATGATTTC	TTCTTTTTTT	540
	AAAATGTCTT	AGGCATATTC					600
	TATTGCATTC	CCGTTTTATT	TTCAAAAGTT	GTTACTGTCT	CTAAATCAAG	AAGAAACCTT	
	CTTAGTAGAT	CCAGCTGATA	TTCAGCCTTT	TTTAAATTGG	ACTGCAGGTT	TTTAAAGGGG	660
45			ATTTCCAGTC	CACACCGTTT	GAGAAGAATA	TACCCGTGAG	720
43	AGCTTCAAGC						780
	TTGCATTAGT	TGTGTGATTA	TACAGTTTTC	TTGTCTTTTT	GCTATGTCCA		
	AGATTCGTGA	AGTTATTAGT	GTAGTCAACG	CATAGGGAGA	GGTGATTGGT	GACTTTTGGA	840
							866
	ՐሮአጥጥጥՐΔՐՐ	TCCTTTTACCC	7""A7""(4				000

	BolJon R200	0 marker					
			GAGATCAGCT	CCAAACATCA	AACAACTTGT	ΔCΔCΔΔΔπδπ	60
	CTTTACTTGC	TAAATGGAAC	ATGACAAGAG	ATAGAAAATC	TTGCTCATAG	TATTGTACAA	120
	GGGATAACAG	TGTAGAAAAC	AAACCGTCTG	TAAGATTTTC	TCCCTGATCC	TCTCACTTAA	180
5	CCAGTAGGCG	TTTTTCACAT	TGAAGCGCAT	ATCTACTTTG	GTATTCACTG	AATAAAAAAA	240
	GAAAGCTGGT	AACATGTGAA	GGATATACAA	GCATTGATAC	ACCAAGTAGT	CACAAACTAC	300
	ATTATAAAGG	TCAGACCTTT	GTTCACATTC	TGGCCTCCAG	GACCACCGCT	TCTAGCAAAG	360
	TTAAGCGTAA	CATGGTCTGC	ACGTATACAA	ATGAAAATGT	TTCTATCAAA	ATCCTATAAA	420
40	ATAGAGCTCT	ATAACATTGT	CGATACATAG	TTTCACTAAC	TCTGCAAGTA	CTAAACACAT	480
10	ATACAAACAA	AACTATGCGA	ACAGATCAAA	ACTACTACAG	AACACAGTTC	TATGACACTG	54C
	TCGATAGTAA	CATCCTCTGC	AAGTACCAAA	GAGATAGCAA	ATGAAACTAT	GTAAACAAAT	60C
	CAAAATTCTA	AATTTCTCCA	TCACAAGGAC	CTACAGAATA	GAGTTATCAT	AACATTTTCT	660
	GTAAATATTT	CCATCAAAAT	GACTAGAGAA	CAGAGTTCTT	ATAACATTAT	CTGTAAATGT	720
4-	TCCAACAAAA	CCACTACATA	GCAGAGTTCT	TATAACATTG	TCTGTAAATG	TCCAATCAAA	780
15	ACCACTACAG	AACAAAGCTC	CTATAACATT	GTTTATACAA	AGTTTCACTA	AATCTACAAA	840
	CTTTCCCCGT	AAATGAGCTT	AATATCACCC	AAAGATGTTT	CAATCAGATA	AAGAGTACGA	900
	CATCGTTTTG	AGATTAGAAC	AAACTGAAAC	TTACGTAGAG	TGATTTGAGG	AGTAGGC	957
	CD410T DOO	.00 1					
20	CP418L R20						
20	AATTTCTCCA	TCACAAGGAC	CTACAGAATA	GAGTTATCAT	AACATTTTCT	GTAAATATTT	60
	CCATCAAAAT	GACTAGAGAA	CAGAGTTCTT	ATAACATTAT	CTGTAAATGT	TCCAACAAAA	120
	CCACTACATA	GCAGAGTTCT	TATAACATTG	TCTGTAAATG	TCCAATCAAA	ACCACTACAG	180
	AACAAAGCTC	CTATAACATT	GTTTATACAA	AGTTTCACTA	AATCTACAAA	CTTTCCCCGT	240
25	AAATGAGCTT	AATATCACCC	AAAGATGTTT	CAATCAGATA	AAGAGTAACG	ACATCGTTTT	300
23	GAGATTAGAA	CAAACTGAAA	CTTACGTAGA	GTGATTTGAG	GAGTAGGCTC	GTTGCCAGCA	360
	ACCURACE	TCTCCTCCGC	CTCATGAAGC	ATCTGTTGCA	CCTGAGACAA	CCGTGACGAA	420
	COCACOCAA	CACCGCCACC	AGAATTCGAC	GCCGCGCATC	GGAAGGATCC	GAATCGGGAA	480
	THURCERCEN	ACAGEGATE	CCGGGAGTGC	GACGGAGCGA	TGGGAAAAGA	GAGTGGCACG	
30	TCTTCCTCCT	CAMPCCCCCC	AGGAGAGGGT	GGTGGATAAA	CTCGCGTATG	ATCAAGTTCG	600
50	ATGTCAATGA	AC GCCGCC	ATTITITIG	TCAGGGCGCT	CTGTGGCTTA	GAAGTTTCCG	
	HIGIOMIGH	AC				•	672
	In the annexe	ed drawing the	t follows the	following abb	revietione ere	. heart	
		a alamina an	10110 113, 1110	tonowing acc	Actiauoits atc	useu.	
	Dra		D	rakkar			
35	Rel-15-1, E3	8 R 1 S	n	2000			
30	101-15-1, 155	0,1(1)	K	2000			
	Hete, Hel, R2	211.Drakkar	he	eterozygous R	211*Drakkar,		
					•	•	

Darm Darmor

Bol: Brassica oleracea

Bra, B.rap: Brassica rapa

**4**0 GCPA18-A19, Wes, Aust: Wesroona

Sam, SamlPGIolSunt5 Samourai

RRH1, ba2c RRH1

rav, N.WR Hybrid Brassica napus\*wild Radish

- Figure 1 illustrates Gamma ray Iradiation and F2 production.
- Figure 2 illustrates seed set on 'R211' and 'R2000'.
  - Figure 3 illustrates the number of seeds per pod of different lines.

- <u>Figure 4</u> illustrates PGIol primer localisation on the segment of PGI sequence from Data Base. In that figure:

PGIol:

- primer PGIol U (named in SGAP: BnPGIch 1 U)

- primer PGIol L (named in SGAP: Bn PGIch 1 L)

5 PGIint:

- primer PGIint U

- primer PGIint L (is out side the sequence).

- <u>Figure 5</u> illustrates electrophoresis gel of PGI-2 gene (PGIol), PCR marker and SG34, a PCR marker close to Rfo.
- Figure 6 illustrates Pgi-2 segment of DNA amplified by PCR with PGIol primers.
- 10 Figure 7 illustrates digestion of the PCR product PGIol by Mse1.

In that figure:

Sam and Darm has a 75bp band.

Drak, R211.Dk and R2000 showed a 70pb one (Acrylamide 15%).

8 was similar to Samourai (75bp); mix with Drakkar (70pb) it allowed the visualisation of the two bands.

- Figure 8 illustrates electrophoresis agarose gel of PGIUNT marker.

In that figure:

PGIUNT band (about 980bp) is present in B.oleracea, B.rapa cv Asko, maintainer and restored lines except in 'R211'.

20 There is no amplification in radish and Arabidopsis.

In various Brassica genotypes only one band was amplified. Size band are similar but sequences are different.

- Figure 9 illustrates electrophoresis gel of PGIint PCR marker.

In that figure PGIint of radish line 7 is of about 950bp. This band is the same as in the restored RRH1 and R113. It is not found in R211. It is not either in R2000. However the PGIint band is of a similar size of about 870bp in the various Brassica species, but sequences are different.

- Figure 10 illustrates electrophoresis agarose gel of BolJon PCR marker.
- Figure 11 illustrates electrophoresis agarose gel of CP418 marker.
- 30 In that figure, the CP418 band (of about 670bp) is specific to the B.oleracea genome. It is present in B.ol, B.napus (Samourai, Drakkar, Pactol and the

herterozygous R2111\*Dk). It is absent from the restored rapeseed (RRH, R113 and R211). It is present in the homozygous R2000.

- Figure 12 illustrates summary markers table.
- Figure 13 (13(a).13(b)) illustrates PGIol marker sequence alignment between Arabidopsis, Radish, B.rapa, B.oleracea and R2000.
  - Figure 14 (14(a), 14(b), 14(c), 14(d)) illustrates the PGlint-UNT marker sequence alignment between Arabidopsis, Radish, B.rapa, B.oleracea and R2000.
  - Figure 15 (15(a), 15(b), 15(c)) illustrates the CP418L marker sequence alignment between Arabidopsis, Radish, B.rapa, B.oleracea and R2000.
- Figure 16 (16(a), 16(b), 16(c), 16(d), 16(e) illustrates Arabidopsis, Radish and B.rapa BolJon markers. There are aligned with DB sequences of Arabidopsis (AC007190end-AC011000beginning), the B.oleracea EMBH959102 end and EMBH448336 begining and representative consensus sequences of the SG129markers band 1 and 2 in B.napus (in Drakkar and Samourai respectively).
- 15 From the point 836bp, AC07190-AC11000 and GCPATpBOJ sequences are no longer closely homologous to the Brassica sequences.
  The radish and B.rapa (GCPconsen RsRf BOJ and BR) sequences are still closely homologous to the B.napus one, from 858bp point to the 900bp and 981 points respectively.
- 20 In radish, only partial homology is found on the Brassica sequence further down.
  In B.rapa species cv Asko, the left of its BolJon sequence can be aligned again, after a 78bp deletion, with those of B.oleracea and B.rapa in B. napus from the 1057bp point to the BolJon L primer.
- <u>Figure 17</u> illustrates the localisation of Pgi-2 primers on the Arabidopsis th 25 MJB21.12 sequence.
  - Figure 18 illustrates the BolJon primers localisation on the mipsAtl62850 gene and overlapping area of AC007190 and AC011000 Arabidopsis th clones. Alignment with the Arabidopsis BolJon PCR product (740bp) is presented.
- It should be understood, however, that the examples are given solely by way of illustration of the object of the invention, of which they in no way constitute a limitation.

Example I: method of producing a double low restorer line of Brassica napus for Ogura cytoplasmic male sterility (cms) presenting a radish introgression, carrying the Rfo restorer gene deleted of the radish Pgi-2 allele and recombined with the Pgi-2 gene from Brassica oleracea, and having a good agronomic value characterised by female fertility, a good transmission rate of Rfo and a high vegetative vigour.

# Materials and methods:

Genotypes: The 'R211' line with a deleted radish insertion was crossed to the spring low GLS rapeseed 'Drakkar' to produce a F1 progeny ('R211\*Dk'). The spring low GLS cms line 'Wesroona' (australian origin) was used for following crosses. Were used as control in molecular analyses: Winter restored lines derived from 'Samourai' carrying the complete ('RRH1') or incomplete ('R113') introgression as well as European radish line7, Asiatic restored radish D81, hybrid Brasica napus\* wild radish, Brassica oleracea, and B.rapa cv Asko, Arabidopsis thaliana.

Gamma ray irradiation: Whole flowering plants were treated with gamma rays from a Co60 source in a controlled area. Subletal dose fo 65 Gray was applied before meioses.

Testcrosses and F2 production: Irradiated plants were transferred in an insectproof greenhouse after removing flower buds larger than 2 mm. The irradiated F1 progeny was used to handpollinate the cms 'Wesroona' line. The restored derived F1' plants were allowed to produce F2 families harvested individually and precisely sown in a field assay along with non irradiated controls (Fig 1).

Phenotypic selection: Three visual criteria were scored (on a 1 to 5 scale) over 2 years in field assays, on 1200 F2 offsprings plus 44 controls (82 330 quoted plants):

- 1-Vegetative vigour,
- 2- Normality of the ratio of fertile /sterile plants in the F2 segregation, and
- 3- Female fertility (pod development and seed set).

Advanced selfed generations of the selected families were obtained either in field or greenhouse and produced homozygous lines (F4) for further analysis.

Isozyme analysis was performed as in (Delourme R. and Eber F. 1992. Theor Appl Genet 85: 222-228), marker development from (Fourmann M et al 2002.

Theor Appl. Genet. 105:1196-1206.): PCR products are validated by sequencing. Alignments were made using Blast Ncbi and Uk Crop Net Brassica DB and the Multialin software INRA Toulouse.

#### Method:

- We choose one low GLS spring homozygous restorer line, 'R211', already exhibiting deletions in the introgression (Delourme R. and Eber F. 1992. Theor Appl Genet 85: 222-228. Delourme R et al 1998. Theor Appl Genet 97: 129-134. Delourme R. et al 1999. 10th Int. Rapeseed Congress, Canberra.). Several molecular markers are missing on either side of Rfo, such as spATCHIA (Fourmann M et al 2002. Theor Appl. Genet. 105:1196-1206), spSG91 (Giancola S et al 2003 Theor Appl. Genet. (in press)). 'R211' lost the isozyme expression of the Pgi-2 allele of the radish gene but also the one of Pgi-2 allele of B.oleracea genome (1,2). Moreover, the homozygous 'R211' shows linked negative traits such as low vigour and very poor seed set. We hypothesised that these plant lack a rapeseed 15 chromosomal segment. The fertile ratio in F2 progenies derived from this material is lower than expected (64% instead of 75%). We initiated the program from this 'R211' line and tried to force recombination between the Rfo carrying introgression from this deleted line and the rapeseed homologous chromosome from a double low B. napus line.
- 20 Ionising irradiation is known to induce chromosomal rearrangements by double strand breaks followed by aberrant rejoining of the ends. Gamma-ray irradiation was used on a heterozygous F1 derived from the 'R211' line to induce chromosome breaks, just before meiosis, aiming at a recombination of the deleted radish introgression in the rapeseed genome.

#### 25 Results:

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Very few families were at the best score for the three criteria out of 1200 F2 families tested.

Only one, 'R2000', proved to produce a normal ratio of fertile plants per selfed progeny with a stable recovery of good agronomic traits such as a good female fertility, with a normal seed set compared to 'R211' (Fig 2 and 3). This family was obtained from a 6 mn irradiation treatment at a dose flow of 65 Gray per hour. Glucosinolate analysis confirmed its low content.

In figure 2 (Seed set on 'R211' and 'R2000') R2000 showed normal inflorescences, with a normal looking architecture.

In figure 3 (Number of seeds per pod), we observe:

- on the best 'R2000' F4 families in self pollination (Selfings) and in testcrosses
- 5 on 'Pactol' cms line on rapeseed and 'R211' controls.

#### Example II: selection of markers in the Pgi-2 gene

PGI isoenzyme analysis: 'R2000' progeny expressed the rapeseed Pgi-2 allele from B. oleracea genome, originally lost in 'R211'.

- 10 Three PCR markers were defined to characterise the R2000 family compared to the known restorer rapeseed RRH1 and R113.
  - 1) PGIol marker was developed from the BrassicaDB sequences to be specific to the Brassica genome. There is no amplification in radish nor in Arabidopsis th., but only in Brassica, with one 248 bp band.
- 2) PGIint marker amplified a longer part of the Pgi-2 gene, allowing clear distinction between the various tested species Brassica, Raphanus and Arabidopsis. The species B.rapa and B.oleracea were not distinguished by the band size on agarose gel, but by their PGINT band sequence.
- 3) PGIUnt marker, a combination of the PGI of U and PGI int L primers.20 This marker had the specificity of the PGIoI marker but amplifying a longer part as for PGIint one.

#### II.1 PGIol marker

25

With the PGIol primers, the 'R211' parental line showed no amplification, while the spring tested lines showed a 248bp band. Its DNA sequence is homologous to the PGI-2 sequences from the Crop Net UK DB in Brassica species and from previous work in our group (named SGAP sequences) (Localisation of the primers SG PGI chou, Fig 4).

It was ortholog of the clone MJB21-12, on the chromosome V, (34543bp) in Arabidopsis (NCBI DB).

30 PGIol plus SG34 to set an Homozygocity test:

The combined use of two sets of primers in a mix PCR, PGIol marking the Pgi-2 gene absent in the homozygote restored plant and SG34 (from S. Giancola et al,

Giancola S et al 2003 Theor Appl. Genet. (in press)), a very close marker to the Rfo gene, was set up to discriminate homozygous from heterozygous plant among the fertile plants segregating in F2 progenies derived from 'R211'. In place of using SG34, it is possible to use any other marker close to or in the Rfo gene.

5 Only one family R2000 showed no difference between homozygote and heterozygote offsprings:

The Pgi-2 gene is present in the R2000 homozygote, which is not the case for the parental homozygous R211.

In figure 5 (PGIol and SG34 PCR markers):

10 The homozygous 'R2000' family has recovered the PGIol band.

DNA sequence of the band confirmed the homology with the known Arabidopsis and Brassica Pgi-2 sequence. Control genotypes (Drakkar, Pactol, and, Samourai, Darmor) had the same pattern on the gel. Sequence of this common band allowed to confirm their high homology as they were quasi similar except one base

The homozygous 'R2000' family has recovered the PGIol band of the Brassica oleracea type. It was distinct from the known restorer of the Samourai group.

This amplified part of the Pgi-2 is very conserved and hardly any differences were shown among the various genotypes. A longer part of Pgi-2 gene was investigated.

#### 20 <u>II.2 PGIUNT and PGlint markers</u>

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substitution.

Electrophoresis Patterns of PCR products:

PGIUNT marker: A second reverse primer, PGIint L, was designed further down the Pgi-2 sequence, to amplify as well conserved and as variable regions of the gene. When used with the PGIol U primer, it amplifies a 980bp band only in Brassica genomes.

R211 didn't show any band, The homozygous 'R2000' showed the PGIUNT band as in the Drakkar parent.

In figure 8 (PGIUNT marker):

PGIint marker amplified a segment of PGIUNT. The upper primer PGIint allows the amplification in all tested species, allowing a clear distinction between Arabidopsis, Radish and Brassica. B.rapa and B.oleracea were not distinguished by the band size on agarose gel, but by their PGIint sequence. All tested restored genotypes, but the

'R211' line, exhibited the European radish band and one Brassica band, homologous to the B.rapa one.

The homozygous 'R2000' didn't show the radish PGIint band, as in the deleted 'R211' parental line, but showed one Brassica band, homologous to the B. oleracea one.

Electrophoresis of PGIint marker is represented in figure 9.

Sequence analysis:

Comparison of the PGI sequences from the data bases.

A PGI segment of about 490bp is known.

Sequences of a segment of about 490bp from different genotypes (B. oleracea, B. rapa, B. napus) have been studied in our laboratory group and some sequences were given to Brassica Crop Net DB: EMAF25875 to 25788 by M.Fouramnn (4) These sequences are very conserved.

Comparison of the B. rapa et B. oleracea species PGI sequences (figures 13 and 14):

Comparison between PGI sequences we have obtained from the tested genotypes of B.oleracea and B.rapa species, showed that they were distinct by 21 base substitutions. Theses substitutions allowed to distinguish PGIint sequences from the other tested genotypes of rapeseed, homologous to either B.rapa cv Asko (RRH1 and R113) or B.oleracea (Drakkar, R211\*DK but also R2000).

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# Example III: selection of marker in a region close to Rfo

Markers surrounding the Rfo gene in the radish insertion were determined in order to facilitate the Rfo gene cloning (Desloires S et al 2003 EMBO reports 4, 6:588-594). One of these, the SG129 PCR marker was located very close to Rfo (Giancola S et al 2003 Theor Appl. Genet. (in press)): it co-amplified distinct bands in B.oleracea and B.rapa genomes of B.napus, but the radish band was very difficult to see on an agarose gel.

The target SG129 sequence was ortholog of a clone (AC011000, at the locus F16P17) in Arabidopsis thaliana. This clone overlapped an Arabidopsis adjacent contig clone (AC07190).

From the Brassica Crop Net DB, we found one B.oleracea clone, (EMBH448336, 764bp) blasting with the beginning of the A011000, and a second B.oleracea clone...

(EMBH53971), distant from about 300bp on the Arabidopsis map, that blasted with the end of ACO7190.

We designed a new PCR marker, BolJon, between the two B.oleracea clones. We verified that it allowed amplification of a specific PCR bands in the different genotypes compared here.

In figure 16 (electrophoresis gel of BolJon PCR products):

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- In Arabidopsis, a BolJon 815bp band was amplified, homologue to the overlapping segment of the contigs.
- In Brassiceae diploid species, BolJon marker showed distinct bands: one of 950bp in B.oleracea and one of 870bp in B.rapa. It showed that the two B.oleracea clones (EMBH53971 and EMBH448336) are in sequence continuity in Brassica genome as it is for the ortholog sequences in Arabidopsis.
  - In B.napus, these two bands are co-amplified in the maintainer lines, Samourai or Drakkar.
- 15 In radish line7, one BolJon band was amplified of about 630 bp long. The band of the restored radish cmsRd81 was slightly smaller.
  - In all the restored rapeseed lines, one of the BolJon bands was of the same size as the radish line7. BolJon is a marker of the radish introgression.
- The homozygous restored rapeseed lines, 'RRH1', 'R113' and also 'R211', only showed the B.rapa band and the 630bp radish band bp suggesting the B.oleracea ortholog of the target gene is absent or has been modified when the radish segment of chromosome was inserted into the rapeseed B.oleracea constitutive genome.

'R2000' homozygote plants showed radish PCR BolJon, plus the two Brassica 25 BolJon bands, again having recovered the B.oleracea one, lost in 'R211' and other restorer lines.

We designed a primer, pCP418L, specific of the B.oleracea genome in the tested species. With the SG129U primer it amplified only one PCR band (670bp) in the B.oleracea species. (Fig 17)

There was no amplification in B.rapa, in radish, nor in Arabidopsis, but there was a clear CP418 band in B. napus maintainer lines. Its sequence was strictly homologous to the EMBH448336 sequence. This marker was in a very conserved

DNA sequence allowing no polymorphism between genotypes except by presence / absence.

In RRH1, R113 and in R211 there was no CP418 band, indicating as previously that the B.oleracea ortholog of the target gene is absent or has been modified following the radish insertion.

'R2000' homozygote plants showed CP418 band, again having recovered the specific B.oleracea one.

In the present invention, a new recombined low GLS restorer line has been selected with a good female fertility. The poor value of line 'R211' allowed selection in the field for a rare recombination event and characterisation the 'R2000' family.

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The homozygous 'R2000' presents a unique combination of the PGIol, PGIUNT, PGIint and BolJon markers when compared with the rapeseed restorer analysed yet: PGIinT marker showed that the homozygous restored rapeseed lines, RRH1 and R113 presented the European radish band plus one Brassica band, homologous to B.rapa genome. 'R2000' shows no radish band, lost as in its parental deleted line R211, but showed one Brassica band homologous to B.oleracea. The ortholog PGIint sequence in its B.rapa genome is not amplified with this marker in R211 and Drakkar genetic background.

PGIol marker and PGIUNT marker sequences in restored lines RRH1 and R113 were homologous to the B.rapa cv Asko one. In 'R2000', PGIUNT sequence is homologous to B.oleracea. The ortholog PGIUnt sequence in its B.rapa genome is not amplified with this marker in R211 and Drakkar genetic background.

BolJon marker showed that the homozygous restored rapeseed lines, including 'R211' presented the European radish band plus only the B.rapa one. 'R2000'

shows the two bands of 'R211' plus the recovered B.oleracea BolJon band.

CP418 marker showed that 'R2000' recovered this conserved B.oleracea segment.

Our hypothesis is that a recombination event took place in the pollen mother cell which gave rise to 'R2000' plants. The deleted radish introgression was then integrated to the normal homologous chromosome segment, carrying the B.oleracea type Pgi-2 gene and BolJon target sequence, characterised by these markers,

type Pgi-2 gene and BolJon target sequence, characterised by these markers, probably from the Drakkar '00' genome present in the irradiated heterozygous 'R211\*DK'.

The pattern observed for BolJon suggests that the recombination event resulted in a particular duplicated region, one from radish and one B.oleracea, in the 'R2000' family.

#### **CLAIMS**

- A method of producing double low restorer lines of Brassica napus for Ogura cytoplasmic male sterility (cms) presenting radish introgression carrying the Rfo restorer gene deleted of the radish Pgi-2 allele and recombined with the Pgi-2 gene from Brassica oleracea, and having a good agronomic value characterised by female fertility, a good transmission rate of Rfo and a high vegetative vigour, said method including the step of:
  - a) crossing double low cms lines of spring Brassica napus comprising a deleted radish insertion with the double low line of spring Drakkar for forming heterozygous restored plants of Brassica napus,
  - b) irradiating before meiosis the heterozygous restored plants obtained in step a) with gamma ray irradiation,
  - c) crossing pollen from flowers obtained in step b) with the cms double low spring Wesroona line,
  - d) testing the progeny for vigour, female fertility and transmission rate of the cms gene,
  - e) selecting progeny lines.
- 20 2. A method according to claim 1, wherein the irradiation dose in step b) is 65 Gray during 6 mn.
  - 3. A method according to claim 1 wherein the double low cms line of spring Brassica napus of step a) is R211.

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- 4. A method according to claim 1 wherein the testing is performed with the combination of five markers selected from PGIol, PGIUNT, PGIint, BolJon and CP418.
- 30 5. Double low restorer lines of Brassica napus for Ogura cytoplasmic male sterility (cms) presenting a Rfo insertion deleted of the radish Pgi-2 allele and recombined with the Pgi-2 gene from Brassica oleracea, and having a

good agronomic value characterised by female fertility, a good transmission rate of Rfo and a high vegetative vigour.

- Double low restorer lines of Brassica napus according to claim 5, wherein
   they present a unique combination of five markers selected from PGIol,
   PGIUNT, PGIint, BolJon and CP418.
  - 7. Brassica napus hybrid plants and progeny thereof obtained through the steps of:
  - a) providing a restorer line produced according to claim 1 and bred to be homozygous,
    - b) using said restorer line in a hybrid production field as the pollinator,
    - c) using cms sterile plants in a hybrid production field as the hybrid seed producing plant, and
- 15 d) harvesting the hybrid seed from the male sterile plant.
  - 8. The seeds of Brassica plant developed from the Brassica line obtained in claim 1.
- 20 9. The seeds of Brassica napus obtained in claim 7.

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10. The seeds of Brassica napus obtained in claims 1 and 2 deposited in NCIMB Limited, 23 St Machar Drive, Aberdeen, Scotland, AB24 3RY, UK, on July 4, 2003, under the reference number NCIMB41183.

11. Use of the combination of at least four markers PGIol, PGIint, BolJon and CP418, or any portion of them comprising at least one polymorphic site, for characterising recombined restorer lines of Brassica napus for Ogura cms presenting a Rfo insertion deleted of the radish Pgi-2 allele and recombined with the Pgi-2 gene from Brassica oleracea, and having a good agronomic value characterised by female fertility, a good transmission rate of Rfo and a high vegetative vigour.

12. Use according to claim 11 wherein the combination is of five markers PGIol, PGIUNT, PGIint, BolJon and CP418.

# 5 13. Use according to claim 12, wherein:

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The marker PGIol is amplified using the primers: PGIol U and PGIol L (PGIol U: 5'TCATTTGATTGTTGCGCCTG3';

PGIol L: 5'TGTACATCAGACCCGGTAGAAAA3')

- The marker PGIint is amplified using the primers: PGIint U and PGIint L (PGIint U: 5'CAGCACTAATCTTGCGGTATG3';
- PGlint L: 5'CAATAACCCTAAAAGCACCTG3')
  - The marker PGIUNT is amplified using the primers: PGIol U and PGIint L: (PGIol U: 5'TCATTTGATTGTTGCGCCTG3';

PGlint L: 5'CAATAACCCTAAAAGCACCTG3')

- The marker BolJon is amplified using the primers: BolJon U and BolJon L: (BolJon U: 5'GATCCGATTCTTCTCTGTTG3';

BolJon L: 5'GCCTACTCCTCAAATCACTCT3')

- The marker CP418 is amplified using the primers: SG129 U and pCP418 L: (SG129 U: cf Giancola et al (5)
- 20 pCP418 L: 5'AATTTCTCCATCACAAGGACC3')

# 14. PGIol marker whose sequence follows:

TCATTTGATT GTTGCGCCTG TCGCCTTGTT GTGTTATGAT GAATGAACAG CAGTCATTTA 60
ACATGTGGTT AACTTAACAG GGCTCCGGCT GTTGCAAAAC ACATGGTTGC TGTCAGCACT 120
AATCTTGCGG TATGAATTTG TGATTAAATT TGTTTGTTTG TGACTCTTTC TTCATTGTTC 180
GTTTTCGTAC AATAAACCGA ATGTATAATC TTTTTACAAA CTGAATTTTC TACCGGGTCT 240
GATGTACA

# 15. PGIUNT marker whose sequence follows:

35	ACATGTGGTT AATCTTGCGG GTTTTCGTAC ATGTACAATG ACAGTACAGA TCGGCATTGA GTAAGTGCTT AACTGAAATT	AACTTAACAG TATGAATTTG AATAAACCGA CTAGTCTCCA AGAAAGTGTT TCCGAACAAT GTTTATTTGG CTTTTGCAGT	GGCTCCGGCT TGATTAAATT ATGTATAATC TGTTCTTGGG CAAAACTCTG GCATTTGCAT TTGTATAAAT TTGCAGTGCT	GTTGCAAAAC TGTTTGTTTG TTTTACAAAC GATCATGATT GATGTTTTAA TTTGGGACTG TTCTCGTCCA GTTGGAGTCT	ACATGGTTGC TGACTCTTTC TGAATTTTCT TATTTTCTAC TTTACAGTTA GGTTGGTGGA TTTCCGCTTG	CAGTCATTTA TGTCAGCACT TTCATTGTTC ACCGGGTCTG ATGTATTCAG GTGGAGAAGT AGGTACAGTG CTTAGTGTAT TCTACAGTAT CATAAAATGT	120 180 240 300 360 420 480 540
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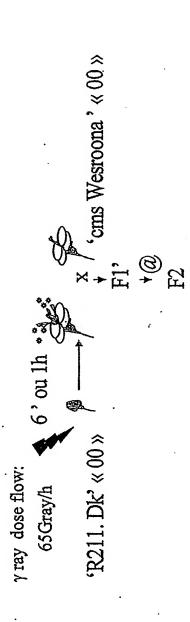
	CTTAGGCATA TTCTTTCTAT TTTAT TTCCCGTTTT ATTTTCAAAA GTTGT GATCCAGCTG ATATTCAGCC TTTTT AGCATTGAAA AGCATTTCCA GTCCA AGTTGTGAA TTATACAGTT TTCTT TGAAGTTATT AGTGTAGTCA ACGCA AGGTGCTTTA GGGTTATTG	TTACTG TCTCTAAATC TTAAAT TGGACTGCAG ACACCG TTTGAGAAGA TGTCTT TTTGCTATGT	AAGAAGAAAC ( GTTTTTAAAG ( ATATACCCGT ( CCATCAACAC !	CTTCTTAGTA GGGAGCTTCA GAGTTGCATT FAGAGATTCG GGACGATTTC	720 780 840 900
	16. PGlint marker whose sequ	uence follows:			
10	CAGCACTAAT CTTGCGGTAT GAAT	ITGTGA TTAAATTTGT	TTGTTTGTGA	CTCTTTCTTC	60
	ATTGTTCGTT TTCGTACAAT AAACC	CGAATG TATAATCTTT	TACAAACTGA	ATTTTCTACC	120
	GGGTCTGATG TACAATGCTA GTCTCTATTCAGACA GTACAGAAGA AAGTC	CCATGT TCTTGGGGAT	GTTTTAATTT	TTTCTACATG ACACTTACTC	180 240
	GAGAAGTTCG GCATTGATCC GAAC	BITCAR ARCICIGGAT	GGGACTGGGT		
15	TACAGTGGTA AGTGCTTGTT TATT	TGGTTG TATAAATTTC	TCGTCCATTT		
10	AGTGTATAAC TGAAATTCTT TTGC	AGTTTG CAGTGCTGTT	GGAGTCTTAC	CATTGTCTCT	420
	ACAGTATGGC TTCTCTGTGG TTGA	GAAGTA CGGTACCTTC	TACTTTATCA		
	AAAATGTCTT AGGCATATTC TTTC TATTGCATTC CCGTTTTATT TTCA	TATTTT ATTTCCCTCT	TAATGATTIC	TICITITIT	540
20	TATIGCATIC CCGITTIATI TICA CTTAGTAGAT CCAGCTGATA TICA	CCCTTT TTACTGICI	ACTGCAGGTT	TTTAAAGGGG	660
20	AGCTTCAAGC ATTGATAAGC ATTT	CCAGTC CACACCGTTT	GAGAAGAATA	TACCCGTGAG	720
	TTGCATTAGT TGTGTGATTA TACA	GTTTTC TTGTCTTTTT	GCTATGTCCA	TCAACACTAG	780
	AGATTCGTGA AGTTATTAGT GTAG	TCAACG CATAGGGAGA	GGTGATTGGT	GACTTTTGGA	840
	CGATTTCAGG TGCTTTAGGG TTAT	TG			866
25					
	17. BolJon marker whose see	quence follows:	•		
	GATCCGATTC TTCTCCTGTT GAGA	TCAGCT CCAAACATCA	AACAACTTGT	ACACAAATAT	60
	CTTTACTTGC TAAATGGAAC ATGA	ACAAGAG ATAGAAAATC	TTGCTCATAG	TATTGTACAA	120
30	GGGATAACAG TGTAGAAAAC AAAC CCAGTAGGCG TTTTTCACAT TGA	CGTCTG TAAGATTTTC	CTATTCACTG	ADTABABABA	240
30	GAAAGCTGGT AACATGTGAA GGAT	PATACAA GCATTGATAC	ACCAAGTAGT	CACAAACTAC	300
	ATTATAAAGG TCAGACCTTT GTTC	CACATTC TGGCCTCCAG	GACCACCGCT	TCTAGCAAAG	360
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٥-	ATAGAGCTCT ATAACATTGT CGA	PACATAG TTTCACTAAC	TCTGCAAGTA	CTAAACACAT	480 540
35	ATACAAACAA AACTATGCGA ACAC TCGATAGTAA CATCCTCTGC AAG	GATCAAA ACTACTACAG	AACACAGTTC	CTANGACACIG	600
	CAAAATTCTA AATTTCTCCA TCAG	CAAGGAC CTACAGAATA	GAGTTATCAT	AACATTTTCT	660
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	TCCAACAAAA CCACTACATA GCA	GAGTTCT TATAACATTO	TCTGTAAATG	TCCAATCAAA	. 780
40	ACCACTACAG AACAAAGCTC CTA CTTTCCCCGT AAATGAGCTT AAT	TAACATT GTTTATACAA	AGTTTCACTA	AATCTACAAA	900
	CATCGTTTTG AGATTAGAAC AAA	ATCACCC AAAGAIGIII CTGAAAC TTACGTAGAG	TGATTTGAGG	AGTAGGC	957
	18. CP418 marker whose se	quence follows:			
45	5 AATTTCTCCA TCACAAGGAC CTA	CAGAATA GAGTTATCAT	AACATTTTCT	GTAAATATTT	60
10	CCATCAAAAT GACTAGAGAA CAG	AGTTCTT ATAACATTA	r ctgtaaatgt	TCCAACAAAA	120
	CCACTACATA GCAGAGTTCT TAT	AACATTG TCTGTAAAT	F TCCAATCAAA	ACCACTACAG	180
	AACAAAGCTC CTATAACATT GTT AAATGAGCTT AATATCACCC AAA	TATACAA AGTTTCACTA	A AATCTACAAA	CTTTCCCCGT	240
50	AAATGAGCTT AATATCACCC AAA GAGATTAGAA CAAACTGAAA CTT	GATGTT CAATCAGAD DCCTDCD GTGDTTTGA	A MAGAGIAACG G GAGTAGGCTC	GTTGCCAGC	360
50	GAGCTAGCTC TCTCCTCCGC CTC	ATGAAGC ATCTGTTGC	A CCTGAGACAA	CCGTGACGA	420
	ACTITCCGAT CACCGCCACC AGA	ATTCGAC GCCGCGCAT	C GGAAGGATCC	: GAATCGGGAA	<b>1 480</b>
	CTGAGTGAAC CCGAGCGATC CCG	GGAGTGC GACGGAGCG	A TGGGAAAAGA	A GAGTGGCACO	3 540
55	ATTTCGACGA AGAGTGGAAG AGG 5 TCATCGTCCT GATTGCCGCC ATT	BAGAGGGT GGTGGATAA	A CTCGCGTATC T CTCTCCCTTT	ATCAAGTTCC A CAACTTTCC	, 660 , 660
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# Method of producing double low restorer lines of Brassica napus having a good agronomic value

## **ABSTRACT**

A method of producing double low restorer line of Brassica napus for Ogura cytoplasmic male sterility (cms) presenting radish introgression carrying the Rfo restorer gene deleted of the radish Pgi-2 allele and recombined with the Pgi-2 gene from Brassica oleracea, and having a good agronomic value characterized by female fertility, a good transmission rate of Rfo and a high vegetative vigour. A method of forming Brassica napus hybrid seeds and progeny thereof. The seeds of Brassica napus and use of the combined markers PGIol, PGIunt, PGIint, BolJon and CP418 for characterising.





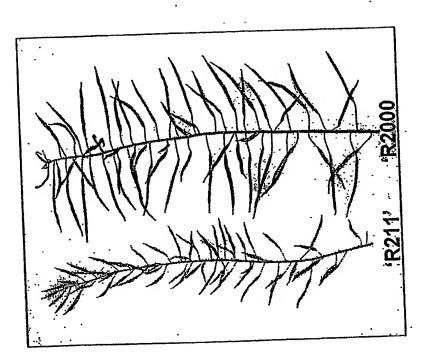
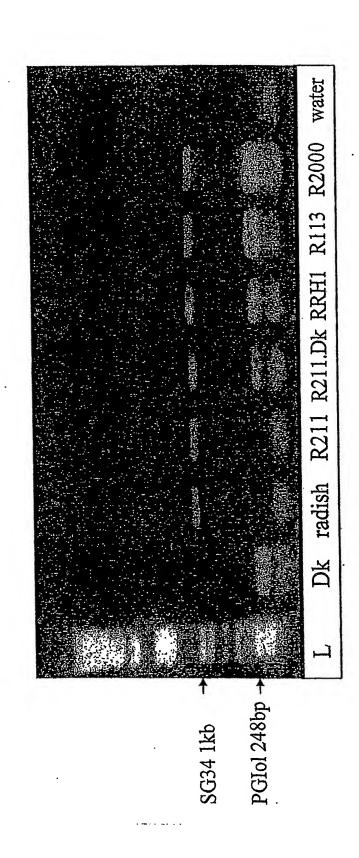


Fig 2

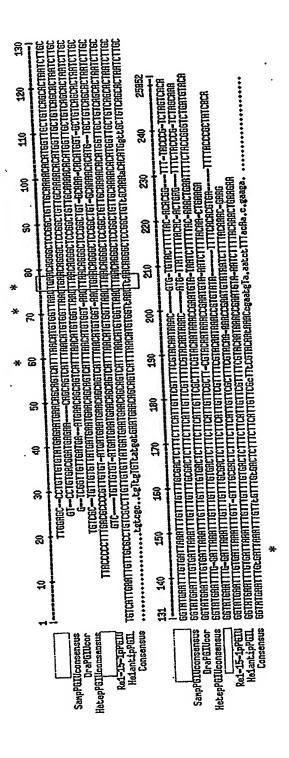
Fig 3

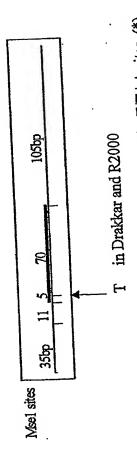
Genotype	Selfings	Test Crosses
Drakkar	29.3	
Pactol	23.1	·
R211	. 11.2	25.5
R2000	26.5 (24.0 – 31.1)	27.0 (24.0 – 28.7)

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Misel restriction enzyme cut DNA sequences at the T/TAA sites (\*)

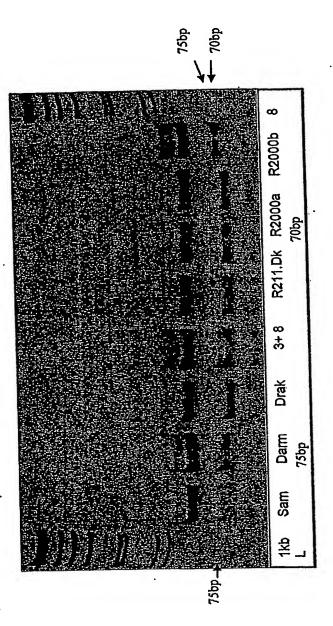
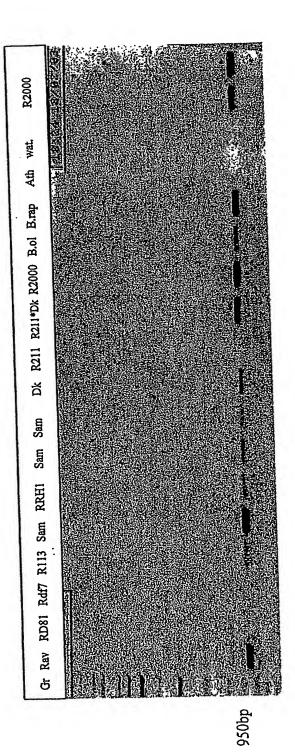
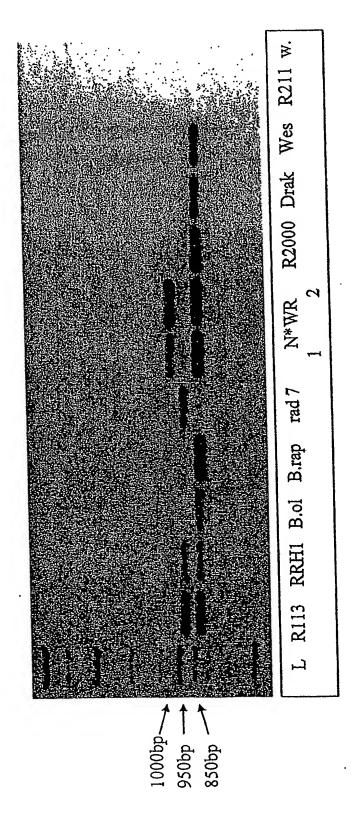


Fig 7

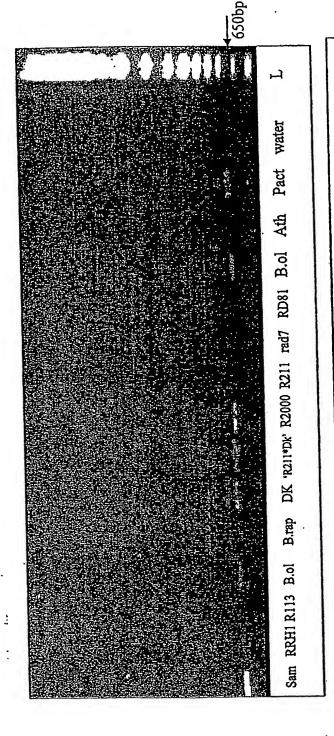








11 11 21 21 11	14 15 10 17						a b c d e	009	290	870	950	800	950 870	0 870			950 870 600	
2.3	11 12 11 13												Pactol	amourai RRH1, 12 R21		11.Dk	E38, 8,18,19 line R1.15	
	4 5 6 7 8 9 10						ds (bp) n°	radish species:line 7	D81 ·	pecies	ea species	osis thaliana (WS)	maintainer rapeseed: 2 Samourai; 3 Pactol	homozygous restored rapeseed: 7 Samourai RRH1, 12 R211	incomplete restored SRfi	heterozygous restored rapeseed: R211.Dk	lozygous restored R2000: 6 lineE38, 8,18,19 line R1.15	ladder1kb+, Ladder 100bp
10	. 7 . 3						BolJon PCR bands	,5 radish sp	7 radish RD81	4 B.rapa species	3 B.olerace	Arabidor	, 3 maintain	12 homozyg	incomple	.8 heterozy	.10 homozye	,L1 ladder11



It is present in B.ol, B.napus (Samourai, Drakkar, Pactol and the herterozygous R211\*Dk) The CP418 band (about 650bp) specific to the B.oleracea genome. It is absent from the restored rapeseed (RRH, R113 and R211) It is present in the homozygous R2000. 12/23

Figure 12

				ŀ					,		*	+			
000	250				•	-	•							+	T
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type D.lap	3								1		+	+	+	•	
-	-				٠	+	+	•	+	١	ı		F		
PGI UNT	980	+	•	- 1						*	<b>1</b>	+	<del>)</del>		
type B. ofe	380			:	i·									+	
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PGI int								7.	THE PROPERTY OF THE PROPERTY O		•			•	
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/anproxim	(annountimative size band)	and)				1	1							_	
Braceira	and is cha	large hand is characterised by its		DNA sequence		_		1	-	+		L	•		

# Figure 13 (a)

	51	81 PGIol U→ 100
consePGIinTUNTDrakka		
consensWesrPGI		••• ••••••
consePGIintUNTR113	• • • • • • • • • • • • • • • • • • • •	••• ••••••• ••• •••••
consePGIintUNTBrapaA ConsePGIintUNTRRH1		
PGIBO-EM: AF258277	TTGCTTAGCG TCCAAAT	TTC ATGATTGTAT TCATTTGATT GTTGTG
PGIBra-EM: AF258278		TTC ATGATTGTAT TCATTTGATT GTTGTGAC
consePGIintUNTBolera		
consePGIintUNTR2000	• • • • • • • • • • • • • • • • • • • •	TTG TCATT.GATTGT.TGCG
Consensus		. 1
	101 5	150
consePGIinTUNTDrakka	101 [>]	TCG TTTGTTGGTG AGT.GAACAG CAGTCATTTA
consensWesrPGI		TTG TGTTATGATG AAT.GAACAG CAGTCATTTA
consePGIintUNTR113		TTGG TAT.GAAACG CAG.CATTTA
consePGIintUNTBrapaA		
ConsePGIIntUNTRRH1		
PGIBo-EM: AF258277 PGIBra-EM: AF258278	<del></del>	GTTG TGTTA.GATG AAT.GAACAG CAGTCATTTA
consePGIintUNTBolera	TAICGCCIC. ICGCCI	CAGICATITA
conserGIIntUNTR2000	/	GTTG TGTTATGATG AAT.GAACA. CAGTCATTTA
Consensus		t.ggt.gaa.ag cagtcattta
		•
	151	MseI restriction site 200
consePGIinTUNTDrakka		AACA GGGCTCCGGC TGTTGCAAAA CACATGGTTG
consensWesrPGI		AACA GGGCTCCGGC TGTTGCAAAA CACATGGTTG AACA GGGCTCCGGC TGTTGCCC CTAAGGGTTG
consePGIintUNTR113 consePGIintUNTBrapaA		ACA GGGCTCCGGC TGTTGCCC CTAAGGGTTG
ConsePGIIntUNTRRH1		AACA GGGC.CCGGC TGTTGCAAACAGTG
PGIBO-EM: AF258277	ACATG.TGGT TAACTT	AACA GGGCTCAGGC TGTTGCAAAA CACATGGTTG
PGIBra-EM:AF258278		AACA GGGCTCCGGC TGTTGCAAAA CATATGGTTG
consePGIintUNTBolera		C TGTTGCAAAA CACATGGTTG
consePGIintUNTR2000		AACA GGGCTCCGGC TGTTGCAAAA CACATGGTTG aaca gggctccggc tgttgcaaaa cacatggttg
Consensus	acaty.tggt taacq.	D D
	001	250
consePGIinTUNTDrakka	201 PGI int U	
consensWesrPGI	CTGT CAGCAC TAATC	
consePGIintUNTR113	CTGT CAGCAC TAATC	
consePGIintUNTBrapaA	CTGT CAGCAC TAATC	TTGC GGTATG AATT TGTGATTAAA TTTGTTTGTT
ConsePGTintUNTRRH1	CTGT CAGCAC TAATC	
PGIBO-EM: AF258277	CTGT CAGCAC TAATO	
PGIBra-EM:AF258278		TTGC GGTATG AATT TGTGATTAAA TTTGTTTGTT
consePGIintUNTBolera	CTGT CAGCAC TAATO	TTGC GGTATG AATT TGTGATTAAA TTTGTTTGTT
consePGIintUNTR2000 Consensus		TTGC GGTATG AATT TGTGATTAAA TTTGTTTGTT ttqc qqtatg aatt tqtgattaaa tttqtttqt
Consensus	·	ctyc gytaty datt tytyattada tttytttyt
	251	300
consePGIinTUNTDrakka	TGTGACTCTT T.CTTC	ATTG TTCGTTTTCG TACAATAAAC CGAATGTATA
consensWesrPGI		ATTG TTCGTTTTCG TACAATAAAC CGAATGTATA
consePGIintUNTR113 consePGIintUNTBrapaA		ATTG TTCGTTTTCG TACAATAAACAATGTATA ATTG TTCGTTTTCG TACAATAAACAATGTATA
ConsergiintUNTRRH1		ATTG TTCGTTTTCG TACAATAAACAATGTATA
PGIBO-EM: AF258277	TGTGACTCTT T.CTTC	ATTG TTCGTTTTCG TACAATAAAC CGAATGTATA
PGIBra-EM: AF258278	TGTGACTCTT TTCTT	ATTG TTCGTTTTCG TACAATAAAC CGAATGTATA
consePGIintUNTBolera		ATTG TTCGTTTTCG TACAATAAAC CGAATGTATA
consePGIintUNTR2000		CATTG TTCGTTTTCG TACAATAAAC CGAATGTATA
Consensus	tg.gactctt t.ctt	cattg ttcgttttcg tacaataaac cgaatgtata
		113

# Figure 13 (b)

consePGIintUNTBrapaA ATCTTTTTAC AAACTGAA consePGIintUNTBolera ATCTTTTAC AAACTGAA Consensus

301 consePGIinTUNTDrakka ATCTTTTTAC AAACTGAA consensWesrPGI ATCTTTTAC AAACTGAA consePGlintUNTR113 ATCTTTTTAC AAACTGAA ConsePGIintUNTRRH1 ATCTTTTTAC AAACTGAA PGIBO-EM: AF258277 ATCTTTTAC AAACTGAA PGIBra-EM:AF258278 ACCTTTTTAC AAACTGAA consePGIintUNTR2000 ATCTTTT.AC AAACTGAA atctttttac aaactgaa

	_	PGIol	ant I.	341	٦ 350
	<				ATGCTAGTC
TT	TTCTACCO	GG TC	rgatg:	PAC A	
TT	TTCTACCO	GG TC	TGATG'	rac a	ATGCTAGTC
TUT	TTCTACCO	GG TC	TGATG'	rac A	ATGCTAGTC
11.7	<b>ጥጥርጥአርርር</b>	CG TC	TGATG'	TAC A	ATGCTAGTC
11.	TICINCC		-0.1 mg	12 C 7	TATE CTACTE
TT					ATGCTAGTC
Terr	TTCTACC	GGG TC	TGATG		ATGCTAGTC
AT		GGG TC	TGATG	TAC F	ATGCTAGTC
			TGATG		ATGCTAGTC
11.1	11011100				
TT	TTCTACC	GGG TC	TGATG	TAC A	ATGCTAGTC
+	t ttctac	caaa t	ctgat	gtac	a atgctAGT

## Figure 14 (a)

201 PGI int U>	250
consePGIinTUNTDrakka	CTGT CAGCAC TAATCTTGC GGTATG AATT TGTGATTAAA TTTGTTTGTT
consensWesrPGI	CTGT CAGCAC TAATCTTGC GGTATG AATT TGTGATTAAA TTTGTTTGTT
consePGIintUNTR113	CTGT CAGCAC TAATCTTGC GGTATG AATT TGTGATTAAA TTTGTTTGTT
consePGIintUNTBrapaA	CTGT CAGCAC TAATCTTGC GGTATG AATT TGTGATTAAA TTTGTTTGTT
ConsePGIintUNTRRH1	CTGT CAGCAC TAATCTTGC GGTATG AATT TGTGATTAAA TTTGTTTGTT
PGIBO-EM: AF258277	CTGT CAGCAC TAATCTTGC GGTATG AATT TGTGATTAAA TTTGTTTGTT
PGIBra-EM: AF258278	CTGT CAGCAC TAATCTTGC GGTATG AATT TGTGATTAAA TTTGTTTGTT
consePGIintUNTBolera	CTGT CAGCAC TAATCTTGC GGTATG AATT TGTGATTAAA TTTGTTTGTT
consePGIintUNTR2000	CTGT CAGCAC TAATCTTGC GGTATG AATT TGTGATTAAA TTTGTTTGTT
Consensus	ctgtcagcac taatcttgc ggtatg aatt tgtgattaaa tttgtttgt
	•
	251 300
consePGIinTUNTDrakka	TGTGACTCTT T.CTTCATTG TTCGTTTTCG TACAATAAAC CGAATGTATA
consensWesrPGI	TGTGACTCTT T.CTTCATTG TTCGTTTTCG TACAATAAAC CGAATGTATA TGCGACTCTT T.CTTCATTG TTCGTTTTCG TACAATAAACAATGTATA
consePGIintUNTR113 consePGIintUNTBrapaA	TGCGACTCTT T.CTTCATTG TTCGTTTTCG TACAATAAACAATGTATA
ConserGiintUNTRRH1	TGCGACTCTT T.CTTCATTG TTCGTTTTCG TACAATAAACAATGTATA
PGIBO-EM: AF258277	TGTGACTCTT T.CTTCATTG TTCGTTTTCG TACAATAAAC CGAATGTATA
PGIBra-EM: AF258278	TGTGACTCTT TTCTTCATTG TTCGTTTTCG TACAATAAAC CGAATGTATA
consePGTintUNTBolera	TG.GACTCTT T.CTTCATTG TTCGTTTTCG TACAATAAAC CGAATGTATA
consePGIintUNTR2000	TGTGACTCTT T.CTTCATTG TTCGTTTTCG TACAATAAAC CGAATGTATA
Consensus	tg.gactctt t_cttcattg ttcgttttcg tacaataaac cgaatgtata
	ε ε3
	301 < PGIol antL 341 350
consePGIinTUNTDrakka	ATCTTTTAC AAACTGAA IT TTCTACCGGG TCTGATGTAC A ATGCTAGTC
consensWesrPGI	ATCTTTTTAC AAACTGAA TT TTCTACCGGG TCTGATGTAC A ATGCTAGTC
consePGIintUNTR113	ATCTTTTTAC AAACTGAA TT TTCTACCGGG TCTGATGTAC A ATGCTAGTC
consePGIintUNTBrapaA	ATCTTTTTAC AAACTGAA TT TTCTACCGGG TCTGATGTAC A ATGCTAGTC
ConsePGIintUNTRRH1	ATCTTTTTAC AAACTGAA TT TTCTACCGGG TCTGATGTAC A ATGCTAGTC
PGIBO-EM: AF258277	ATCTTTTAC AAACTGAA IT TTCTACCGGG TCTGATGTAC A ATGCTAGTC
PGIBra-EM: AF258278	ACCTTTTAC AAACTGAA AT GTCTACCGGG TCTGATGTAC A ATGCTAGTC
consePGIintUNTBolera	ATCTTTTTAC AAACTGAA TT TTCTACCGGG TCTGATGTAC A ATGCTAGTC
consePGIintUNTR2000	ATCTTTT.AC AAACTGAA TT TTCTACCGGG TCTGATGTAC A ATGCTAGTC atcttttac aaactgaa tt ttctaccggg tctgatgtac a atgctAGTC
Consensus	E
	351 400
consePGIinTUNTDrakka	TCCATGTTCT TGGGGATCAT GATTTATTTT CTACATGTAT TCAGACAGTA
consensWesrPGI	TCCATGTTCT TGGGGATCAT GATTTATTTT CT.CATGTAT TCAGACAGTA
consePGIintUNTR113	TCCATGTTCT TGGGGATCAT GATTTATTTT CTACATGTGT TCAGCCAGTA
consePGIintUNTBrapaA	TCCATGTTCT TGGGGATCAT GATTTATTTT CTACATGTGT TCAGCCAGTA
ConsePGIIntUNTRRH1	TCCATGTTCT TGGGGATCAT GATTTATTTT CTACATGTGT TCAGCCAGTA
PGIBO-EM: AF258277	TCCATGTTCT TGGGGATCAT GATTTATTTT CTACATGTAT TCAGACAGTA
PGIBra-EM: AF258278	TCCATGTTCT TGGGGATCAT GATTTATTTT CTACATGTAT TCAGACAGTA
consePGIintUNTBolera consePGIintUNTR2000	TCCATGTTCT TGGGGATCAT GATTTATTTT CTACATGTAT TCAGACAGTA TCCATGTTCT TGGGGATCAT GATTTATTTT CTACATGTAT TCAGACAGTA
Consergiinconikzooo	TCCATGITCT TGGGGATCAT GATTTATTIT CTACATGIAIT TCAGACAGTA TCCATGTTCT TGGGGATCAT GATTTATTTT CTACATGTAT TCAGACAGTA
Consensus	
	ε5 ε6 401 450
consePGIinTUNTDrakka	CAGAAGAAG TGTTCAAAAC TCTGGATGTT TTAATTTACA GTTAGTGGAG
consensWesrPGI	CAGAAGAAAG TGTTCAAAAC TCTGGATGTT TTAATTTACA GTTAGTGGAG
consePGIintUNTR113	
consePGIintUNTBrapaA	
ConsePGIintUNTRRH1	CAGAAGAAG TGTTTAAAAC TCTGGATGTT TTAATTTACA GTTAGTGGAG
PGIBO-EM: AF258277	
PGIBra-EM:AF258278	
consePGIintUNTBolera	
consePGIintUNTR2000	
Consensus	
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#### Figure 14 (b)

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•	end of Data Base PGI sequences 500
nor: -mump - kka	ANGUTCOGCA TIGATOCGAA CAATGCATTI GCATTITGGG ACTGGGTTGG
Consergimionapara	AACTTCGGCA TTGATCCGAA CAATGCATTT GCATTTTGGG ACTGGGTTGG
CONSCIONATE 113	AAGTTCGGCA TTGATCCGAA CAATGCATTT GCATTTTGGG ACTGGGTTGG
Comseroranconaria	PACTURECCEA TEGATECGAA CAATGCATTT GCATTTTGGG ACTGGGTTGG
ConserGIIIICOMIDIAP	AAGTTCGGCA TTGATCCGAA CAATGCATTT GCATTTTGGG ACTGGGTTGG
DOTD - EM- A E259277	ABGTTCGGCA TTGATCC
EGIDO ELITITADOS.	ARCHPOCCCO PTGATCCGAA CAA
consePGIintUNTBolera	ARCHROGGCA TTGATCCGAA CAATGCATTT GCATTTTGGG ACTGGGTTGG
consePGIintUNTR2000	AAGTTCGGCA TTGATCCGAA CAATGCATTT GCATTTTGGG ACTGGGTTGG
Consensus	AAGTTCGGCA TTGATCCgaa caatgcattt gcattttggg actgggttgg
	550
	501
consePGIinTUNTDrakka	
consensWesrPGI	TGGAAGGTAC AGTGGTAAGT GCTTGTTTAT TTGGTTGTAT AAATTTCTCG
consePGIintUNTR113	TGGAAGGTAC AGTGGTAAGT GCTTGTTTAT TTGGTTGTAT TAATTTCTCA
consePGIintUNTBrapaA	TGGAAGGTAC AGTGGTAAGT GCTTGTTTAT TTGGTTGTAT TAATTTCTCA
ConsePGIintUNTRRH1	TGGAAGGTAC AGTGGTAAGT GCTTGTTTAT TTGGTTGTAT TAATTTCTCA
. PGIBO-EM: AF258277	
PGIBra-EM: AF258278	TOTAL ACTICETANCE CONTENTED TIGGTIGTAT AAATTICICG
consePGIintUNTBolera	MULLANIA MICHARIA COLLOCALIA COLL
consePGIintUNTR2000	TGGAAGGTAC AGTGGTAAGT GCTTGTTTAT TTGGTTGTAT AAATTTCTCG
Consensus	tggaaggtac agtggtaagt gcttgtttat ttggttgtat .aatttctc.
	8 9
	CE1
consePGIinTUNTDrakka	TCCATETCCG CTTGCTTAGT GTATAACTGA AATTCTTTTG CAGTTTGCAG
consensWesrPGI	TCCATTTCCG CTTGCTTAGT GTATAACTGA AATTCTTTTG CAGTTTGCAG
consePGIintUNTR113	TCCATATCCG CTTGCTTAGT TTATAACTGA AATTCTTTTG CAGTTTGCAG
consePGIintUNTBrapaA	TCCATATCCG CTTGCTTAGT TTATAACTGA AATTCTTTTG CAGTTTGCAG
ConsePGIintUNTRRH1	TCCATATCCG CTTGCTTAGT TTATAACTGA AATTCTTTTG CAGTTTGCAG
PGIBO-EM: AF258277	• • • • • • • • • • • • • • • • • • • •
PGIBra-EM: AF258278	TCCATTTCCG CTTGCTTAGT GTATAACTGA AATTCTTTTG CAGTTTGCAG
consePGIintUNTBolera	TCCATTTCCG CTTGCTTAGT GTATAACTGA AATTCTTTTG CAGTTTGCAG
consePGIintUNTR2000	TCCATTTTCCG CTTGCTTAGT GTATAACIGA AATTCTTTTC GTGTTTCCG
Consensus	tccat.ltccg cttgcttagt .ltataactga aattcttttg cagtttgcag
	10. 11
	601 TGCTGTTGGA GTCTTACCAT TGTCTCTACA GTATGGCTTC TCTGTGGTTG
consePGIinTUNTDrakka	
consensWesrPGT	
consePGIintUNTR113	
consePGIintUNTBrapaA	
ConsePGIintUNTRRH1	
PGIBO-EM: AF258277	, , , , , , , , , , , , , , , , , , , ,
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consensWesrPG	
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consePGIintUNTBrapa ConsePGIintUNTRRH	1 BCBBCTBCGC TACCTTCTAC TTTATTAGCC ATCTCATAGA AIGICITCGG
PGIBO-EM: AF25827	7
PGIBO-EM: AF25827	A
rGlBra-EM:AF23627 consePGIintUNTBoler	POR CORRECCE MACCOMMONAC TOTATION GCC ATCTCATAAA A.GTCTTAGG
consePGIIntUNTR200	A TOTA CONTINUE TO THE TOTAL THE TOTAL AT CONTINUE TO THE CONT
Consensu	13

# Figure 14 (c)

	701				750
consePGIinTUNTDrakka	CATATTCTTT	CTATTTTATT	TTCCTCTTAA	TGATTTCTTC	THTTTTTTTT
consensWesrPGI			TTCCTCTTAA		TTTTTTTAT
consePGIintUNTR113	CATATTCTTT		TTCCTCTGAA		TCTTTTAT
consePGIintUNTBrapaA			TTCCTCTGAA		TCTTTTAT.
ConsePGIintUNTRRH1	CATATTCTTT	CTATTTTATT	TTCCTCTGAA	TGATTTCTTC	
PGIBO-EM: AF258277	• • • • • • • • •				
PGIBra-EM: AF258278					
consePGIintUNTBolera	CATATTCTTT	CTATTTTATT	TTCCTCTTAA	TGATTTCTTC	יים דיים דיים דיים דיים דיים דיים דיים
consePGIintUNTR2000				TGATTTCTTC	
Consensus				tgatttcttc	
Consensus	Cacaccccc	Ccacoccacc	15	cgaccccccc	16 17
	751		10		800
consePGIinTUNTDrakka		դարա Ծանանան	ΑΑΑΑGΤΤGΤΤ	ACTGTCTCTA	
consensWesrPGI				ACTGTCTCTA	
consePGIintUNTR113				ACTGTCTCTA	
consePGIintUNTBrapaA				ACTGTCTCTA	
ConsePGIintUNTRRH1				ACTGTCTCTA	
PGIBO-EM:AF258277	_				•
PGIBra-EM:AF258278					
consePGIintUNTBolera	TGCATTCCCG			CGGCCCCTA	
consePGIintUNTR2000				ACTGTCTCTA	
Consensus				actgtctcta	
				J	
	801				850
consePGIinTUNTDrakka				AGCCTTTTTT	
consensWesrPGI	AAACCTTCTT	AGTAGATCCA	GGTGATATTC	AGCCTTTTTT	AAATTGGACT
consePGIintUNTR113	AAACCTTCTT	AGTAGATCCA	GTTGATATTC	AGCCTTTTCT	AAATTGGACT
consePGIintUNTBrapaA	AAACCTTCTT	AGTAGATCCA	G.TGATATTC	AGCCTTTTCT	AAATTGGACT
ConsePGIintUNTRRH1	AAACCTTCTT	AGTAGATCCA	GTTGATATTC	AGCCTTTTCT	AAATTGGACT
PGIBO-EM:AF258277					
PGIBra-EM:AF258278	• • • • • • • • • • • • • • • • • • • •				
consePGIintUNTBolera	AAACCTTTCT	AGGACCA	GACTCC	ACCCTTTTTT	AAATTGGACT
consePGIintUNTR2000	AAACCTTCTT	AGTAGATCCA	GCTGATATTC	AGCCTTTTTT	AAATTGGACT
Consensus	aaaccttctt	agtagatcca	g.tgatattc	agcctttt.t	aaattggact
•			18	19	
	851			_	900
consePGIinTUNTDrakka	GCAGGTTTTT	AAA.GGGAGC	TTCAAGCATT	اساب ا	TCCAGTCCCC
consensWesrPGL	GCAGGTTTTT	AAA.GGGAGC	TTCAAGCATT	GATAAGCATT	TCCAGTCCAC
consePGIintUNTR113	GCAGGTTTTT	AAA.GGGAGC	TTCAAGCATT	GATCAGCATT	TCCAGTCC
consePGIintUNTBrapaA	GCAGGTTTTT	AAA.GGGAGC	TTCAAGCATT	GATCAGCATT	TCCAGTCC
ConsePGIintUNTRRH1	GCAGGTTTTT	AAA.GGGAGC	TTCAAGCATT	GATCAGCATT	TCCAGTCCAC
PGIBo-EM: AF258277	• • • • • • • • •				• • • • • • • • •
PGIBra-EM: AF258278	• • • • • • • • • •	• • • • • • • • •			• • • • • • • • • • •
consePGIintUNTBolera					TCCAGTCCAC
consePGlintUNTR2000					TCCAGTCCAC
Consensus	gcaggttttt	aaa.gggagc	ttcaagcatt	gat[]agcatt	tccagtcc.c
				20	
	901				950
consePGIinTUNTDrakka					GTGTGATTAT
consensWesrPGI					GTGTGATTAT
consePGIintUNTR113					GTGATTAT
consePGIintUNTBrapaA					GTGTGATTAT
ConsePGIIntUNTRRH1					GTGTGATTAT
PGIBO-EM: AF258277				• • • • • • • • • •	
PGIBra-EM: AF258278 consePGIintUNTBolera					COCOCADON
consePGIIntUNTBOLETA					GTGTGATTAT
					GTGTGATTAT
Consensus	acc.gtttga	. gaagaatata	a cccgtgagtt	gcattagtt.	gtgtgattat

#### Figure 14 (d)

	951				1000
consePGIinTUNTDrakka	ACAGTTTTTC	TTGTCTTTTT	GCTATGCCCA	TCAACACTAG	AAGATTCGTG
consensWesrPGI	ACAGTTTT.C				A.GATTCGTG
consergiintUNTR113		TTGCCTTTTT		GCAAC.CTAG	
consePGIintUNTBrapaA	ACAGTTTT.C			TCAAC. CTAG	
ConsePGIIntUNTRRH1			GCTATAT	GCAACCCTAG	GATTCATG
PGIBO-EM: AF258277				•••••	
PGIBO EM: NE258278					
consePGIintUNTBolera	አርአርጥጥጥጥ C	ատշտափափ	GCTAG. TGA	TCAAC, CTAG	A.GATTCGTG
consePGIIntUNTR2000					A.GATTCGTG
Consensus	acagtttt.c	ttgtcttttt	gctata	tcaac.ctag	a.gattcltg
					21
	1001				1050
consePGIinTUNTDrakka					GCTGTTTTGG
consensWesrPGI		TGTAGTCAAC			• • • • • • • • • •
consePGIintUNTR113				GTTCACTG	
consePGlintUNTBrapaA				AGG. TGATTG	• • • • • • • • •
ConsePGIintUNTRRH1	AAGTTATTAG	TGTAGTCAAC			
PGIBo-EM: AF258277	• • • • • • • • •				• • • • • • • • • •
PGIBra-EM:AF258278	• • • • • • • • • •			• • • • • • • • •	
consePGlintUNTBolera				AGC	
consePGIintUNTR2000					TGACTTTTGG
Consensus	aagttattag	tgtagtcaac	gca.agg.g.	.g	• • • • • • • • •
			4056		
	1051		1076		~~~~
consePGIinTUNTDrakka	ACGTTTTCAC			GGACCAAACC	CCCAAC
consensWesrPGI	• • • • • • • • • •	• • • • • • • • •		• • • • • • • • •	• • • • • •
consePGIintUNTR113	• • • • • • • • • •	• • • • • • • • • •		• • • • • • • • •	
consePGIintUNTBrapaA	• • • • • • • • • •	• • • • • • • • •			
ConsePGIIntUNTRRH1	• • • • • • • • •	• • • • • • • • •	• • • • • • • • • • • • • • • • • • • •	• • • • • • • • •	
PGIBO-EM: AF258277	• • • • • • • • • •	• • • • • • • • •	• • • • • • • • • •		
PGIBra-EM:AF258278	• • • • • • • • •	• • • • • • • • •		• •	• • • • • •
consePGIintUNTBolera	• • • • • • • • • • •	• • • • • • • • •			
consePGIintUNTR2000	ACGATTT CAG	GTGCTTTAGG	GTTATTG		

# Figure 15 (a)

	400
	51 100
EMBH44836anti	***************************************
GCP18-5CP418L-Sams GCP18-2CP418L-Wes	********************************
GCP18-2CP418L-R2000	CP418L
consel29bal-Drak	AAACAAATCA AAATTCTAAA TTTCTCCA
GCPS18-129Sam-ba2	AAAC TATGTA ACAAAAATCA AAATTGTAAA TGTCTCCA
GCPR18-3129R211-ba2	AA CCAAAAATCC AAATTGTAAA TGTTCCCT.
GCP18-10129R20-ba2	CAAAATCCA AAATTGTAAA TGTC.CCT
Consensus	
	101
EMBH44836anti	
GCP18-5CP418L-Sams	A. CATTTTCT GTAA
GCP18-2CP418L-Wes	
GCP18-4CP418L-R2000	TCACAAGGAC CTACAGAATA GAGTTATCAT AACATTT CT GTAA
consel29bal-Drak	TCGTAACGAC LTACAGAATA GAGTTATCAT AACATTTTCT G AA
GCPS18-129Sam-ba2 GCPR18-3129R211-ba2	TETTANTES CTCAAAA A GAGGTATCAA AAC.TTTTCT GT.A
GCP18-3129R211-ba2	TGGTTACCGC C.CAAAAA.A AAGGTCAA AACTT.TCCG GTAA
Consensus	***************************************
	200
EMBH44836anti	151
GCP18-5CP418L-Sams	TATTICCAT CAAAATGA CTAGAGAAC AGCAGTICTI ATAACATTAT
GCP18-2CP418L-Wes	.TATTTCCAT CAAAATGACTAGAGAAC AG.AGTTCTT ATAACATTAT
GCP18-4CP418L-R2000 consel29bal-Drak	ATATTTCCAT CARACTGA CTAGAGAAC AG.AGTTCTT ATAACATTAT
GCPS18-129Sam-ba2	ATGTTTCCAT CAAAATGA CTATCGAAC ATAATTAAT ATA.CATTTT
GCPR18-3129R211-ba2	ATGTTTCCAT CAAAATG. CTATCGGAC ATAATTAAT ATAAC.TICT ATGTTTCCAT CAAAATG. CTTCGGA.C ATAATTAAT ATAAC.TTCT
GCP18-10129R20-ba2 Consensus	ATGITICCCT CAAA.TGG CTTCGGA.C ATAATTAAA ACATTCT
	250
	201
EMBH44836anti GCP18-5CP418L-Sams	CTGTAAA TG TTCCAA CAAAA CCACT ACATAGCAGAGTTC .TTATAACAT
GCP18-3CP410B 5GM	CTGTAAA TG.TTCCAA CAAAA CCACT ACATAGCAGAGTTC ATTATAACAT
	CTGTAAA TG.TTCCAA CAAAA CCACT ACATAGCAGAGTTC .TTATAACAT
GCP18-4CP418L-R2000	
conse129ba1-Drak	CTGTAAA TG.TTCCAA CAAAA CCACT ACATAGCAGAGTTC .TTATAACAT
conse129ba1-Drak GCPS18-129Sam-ba2	CTGTAAA TG.TTCCAA CAAAA CCACT ACATAGCAGAGTTC .TTATAACAT CTG.AAAATAATTCCCCTCAAAAATTAACATT TTC TTACAA.A.
conse129ba1-Drak GCPS18-129Sam-ba2 GCPR18-3129R211-ba2	CTGTAAA TG.TTCCAA CAAAA CCACT ACATAGCAGAGTTC .TTATAACAT CTG AAAATAATTCCCTCAAAAATTAL .CATT TTC T.ACAA.A. CTG AAAAT ATTCCCT CAAAA TTAL ACATT TTC T.ACAA.A.
conse129ba1-Drak GCPS18-129Sam-ba2 GCPR18-3129R211-ba2 GCP18-10129R20-ba2	CTGTAAA TG.TTCCAA CAAAA CCACT ACATAGCAGAGTTC .TTATAACAT CTG.AAAATAATTCCCTCAAAAATTALCATT TTC T.ACAA.A. CTG.AAAAT.ATTCCCTCAAAAA TTAL. ACATT TTC T.ACAA.A. CTG.AAA.TAATTCCCTCAAAAA TTAL. ACAT. TTC T.ACAA.A.
conse129ba1-Drak GCPS18-129Sam-ba2 GCPR18-3129R211-ba2	CTGTAAA TG.TTCCAA CAAAA CCACT ACATAGCAGAGTTC .TTATAACAT CTG.AAAATAATTCCCCTCAAAAATTACATT
conse129bal-Drak GCPS18-129Sam-ba2 GCPR18-3129R211-ba2 GCP18-10129R20-ba2 Consensus	CTGTAAA TG.TTCCAA CAAAA CCACT ACATAGCAGAGTTC .TTATAACAT CTG.AAAATAATTCCCTCCAAAAATTACATT
conse129bal-Drak GCPS18-129Sam-ba2 GCPR18-3129R211-ba2 GCP18-10129R20-ba2 Consensus	CTGTARA TG.TTCCAA CAARA CCACT ACATAGCAGAGTTC .TTATARCAT CTG.AARATAAATTCCCTCAARAATTALCATT TTC T.ACAA.A
conse129bal-Drak GCPS18-129Sam-ba2 GCPR18-3129R211-ba2 GCP18-10129R20-ba2 Consensus EMBH44836anti GCP18-5CP418L-Sams	CTGTAAA TG.TTCCAA CAAAA CCACT ACATAGCAGAGTTC .TTATAACAT CTG.AAAATAATTCCCCT.CAAAAA TTA. ACATT TTC T.ACAA.A. CTG.AAAA.TAATTCCCT CAAAA TTA. ACAT. TTC T.ACAA.A.  251 300CTATACC TGTCTGT.AA ATGTCCAATC AAAACCACTA CAGAACAAAG CTCCTATAAC TTGTCTGT.AA ATGTCCAATC AAAACCACTA CAGAACAAAG CTCCTATAAC
conse129bal-Drak GCPS18-129Sam-ba2 GCPR18-3129R211-ba2 GCP18-10129R20-ba2 Consensus	CTGTAAA TG.TTCCAA CAAAA CCACT ACATAGCAGAGTTC CTG.AAAATAATTCCCTCCAAAAATTAL. CATT TTC TTACAA.A. CTG.AAAAT.ATTCCCT CAAAA TTAL. ACATT TTC T.ACAA.A. CTG.AAA.TAATTCCCT CAAAA TTAL. ACAT. TTC T.ACAA.A.  251  300 .CTATACC TGTCTGT.AA ATGTCCAATC AAAACCACTA CAGAACAAAG TGTCTGT.AA ATGTCCAATC AAAACCACTA CAGAACAAAG TGTCTGT.AA ATGTCCAATC AAAACCACTA CAGAACAAAG TGTCTGT.AA ATGTCCAATC AAAACCACTA CAGAACAAAG CTCCTATAAC CTCCTATAAC
CONSE129bal-Drak GCPS18-129Sam-ba2 GCPR18-3129R211-ba2 GCP18-10129R20-ba2 CONSENSUS  EMBH44836anti GCP18-5CP418L-Sams GCP18-4CP418L-R2000 CONSE129bal-Dral	CTGTAAA TG.TTCCAA CAAAA CCACT ACATAGCAGAGTTC CTG.AAAATAATTCCCTCAAAAATTAL. CATT TTC CTG.AAAATAATTCCCT CAAAA TTAL. ACATT TTC CTG.AAA.TAATTCCCT CAAAA TTAL. ACAT. TTC T.ACAA.A.  251  300 .CTATACC TGTCTGT.AA ATGTCCAATC AAAACCACTA CAGAACAAAG CTCCTATAAC CTCCTATAAC
CONSE129bal-Drak GCPS18-129Sam-ba2 GCPR18-3129R211-ba2 GCP18-10129R20-ba2 CONSENSUS  EMBH44836anti GCP18-5CP418L-Sams GCP18-4CP418L-R2000 CONSE129bal-Drak GCPS18-129Sam-ba2	CTGTAAA TG.TTCCAA CAAAA CCACT ACATAGCAGAGTTC CTG.AAAATAATTCCCTCAAAAAATTA. CATT TTC CTG.AAAATAATTCCCT CAAAA TTA. ACATT TTC CTG.AAA.TAATTCCCT CAAAA TTA. ACAT. TTC T.ACAA.A.  251  300 .CTATACC TGTCTGT.AA ATGTCCAATC AAAACCACTA CAGAACAAAG TGTCTGT.AAA
CONSE129bal-Drak GCPS18-129Sam-ba2 GCPR18-3129R211-ba2 GCP18-10129R20-ba2 CONSENSUS  EMBH44836anti GCP18-5CP418L-Sams GCP18-2CP418L-Wes GCP18-4CP418L-R2000 CONSE129bal-Drai GCPS18-129Sam-ba2 GCPR18-3129R211-ba2	CTGTAAA TG.TTCCAA CAAAA CCACT ACATAGCAGAGTTC CTG.AAAATAATTCCCTCAAAAAATTA. CATT TTC CTG.AAAATAATTCCCT CAAAA TTA. ACATT TTC CTG.AAA.TAATTCCCT CAAAA TTA. ACAT. TTC T.ACAA.A.  251  300 .CTATACC TGTCTGT.AA ATGTCCAATC AAAACCACTA CAGAACAAAG TGTCTGT.AAA
CONSE129bal-Drak GCPS18-129Sam-ba2 GCPR18-3129R211-ba2 GCP18-10129R20-ba2 CONSENSUS  EMBH44836anti GCP18-5CP418L-Sams GCP18-2CP418L-Wes GCP18-4CP418L-R2000 CONSE129bal-Drai GCPS18-129Sam-ba2 GCPR18-3129R211-ba2 GCP18-10129R20-ba2	CTGTARA TG.TTCCAA CARAA CCACT ACATAGCAGAGTTC CTG.AARATARTCCCTCAARAATTAL. CATT TTC CTG.AARAT.ARTCCCT CARAA TTAL. ACATT TTC CTG.AARA.TAATTCCCT CARAA TTAL. ACATT TTC CTG.AARA.TAATTCCCT CARAA TTAL. ACAT. TTC  251  300 .CTATACC CTGTCTGT.AR ATGTCCARTC ARAACCACTA CAGARCARAG TGTCTGT.AR ATGTCCARTC ARAACCACTA CAGARCARAG TGTTTC
CONSE129bal-Drak GCPS18-129Sam-ba2 GCPR18-3129R211-ba2 GCP18-10129R20-ba2 CONSENSUS  EMBH44836anti GCP18-5CP418L-Sams GCP18-2CP418L-Wes GCP18-4CP418L-R2000 CONSE129bal-Drai GCPS18-129Sam-ba2 GCPR18-3129R211-ba2	CTGTARA TG.TTCCAA CARAA CCACT ACATAGCAGAGTTC CTG ARAATAMTTCCCT CARAR TTA. ACATT TTC CTG ARAAT.ATTCCCT CARAR TTA. ACATT TTC CTG ARAAT.ATTCCCT CARAR TTA. ACATT TTC CTG ARAA.TAATTCCCT CARAR TTA. ACATT TTC CTG ARAA.TAATTCCCT CARAR TTA. ACATT. TTC T.ACAA.A.  300 CTATACC CTGTCTGT.AA ATGTCCARTC ARAACCACTA CAGARCARAG TGTCTGT.AA ATGTCCARTC ARAACCACTA CAGARCARAG TGTTTC
CONSE129bal-Drak GCPS18-129Sam-ba2 GCPR18-3129R211-ba2 GCP18-10129R20-ba2 CONSENSUS  EMBH44836anti GCP18-5CP418L-Sams GCP18-2CP418L-Wes GCP18-4CP418L-R2000 CONSE129bal-Drai GCPS18-129Sam-ba2 GCPR18-3129R211-ba2 GCP18-10129R20-ba2	CTGTAAA TG.TTCCAA CAAAA CCACT ACATAGCAGAGTTC CTG.AAAATAATTCCCT CAAAA TTA. ACATT TTC CTG.AAAAT.ATTCCCT CAAAA TTA. ACATT TTC CTG.AAA.TAATTCCCT CAAAA TTA. ACAT. TTC T.ACAA.A.  251  300 .CTATACC TGTCTGT.AA ATGTCCAATC AAAACCACTA CAGAACAAAG TGTTTC
CONSE129bal-Drak GCPS18-129Sam-ba2 GCPR18-3129R211-ba2 GCP18-10129R20-ba2 CONSENSUS  EMBH44836anti GCP18-5CP418L-Sams GCP18-4CP418L-R2000 CONSE129bal-Dral GCPS18-129Sam-ba2 GCPR18-3129R211-ba2 GCPR18-10129R20-ba3 CONSENSUS	CTGTARA TG.TTCCAA CARAA CCACT ACATAGCAGAGTTC CTG.AAAATAATTCCCTCAAAAATTAL. CATTT TTC CTG.AAAAT.ATTCCCT CAAAA TTAL. ACATT TTC CTG.AAAA.TAATTCCCT CAAAA TTAL. ACATT. TTC T.ACAA.A.  251  300CTATACC CTGTCTGT.AA ATGTCCAATC AAAACCACTA CAGAACAAAG TGTCTGT.AA ATGTCCAATC AAAACCACTA CAGAACAAAG TGTTTC
CONSE129bal-Drak GCPS18-129Sam-ba2 GCPR18-3129R211-ba2 GCP18-10129R20-ba2 CONSENSUS  EMBH44836anti GCP18-5CP418L-Sams GCP18-5CP418L-R2000 CONSE129bal-Dral GCPS18-129Sam-ba2 GCPS18-129Sam-ba2 GCPR18-3129R211-ba2 GCPR18-3129R211-ba2 GCPR18-10129R20-ba2 CONSENSUS	CTGTARA TG.TTCCAA CARAA CCACT ACATAGCAGAGTTC CTG ARAATAMTTCCCT CARAR TTA. ACATT TTC CTG ARAATAMTTCCCT CARAR TTA. ACATT TTC CTG ARAATAMTCCCT CARAR TTA. ACATT TTC CTG ARAA.TAATTCCCT CARAR TTA. ACATT. TTC CTCTATACC CTCTATACC CTCCTATACC CTC
CONSE129bal-Drak GCPS18-129Sam-ba2 GCPR18-3129R211-ba2 GCP18-10129R20-ba2 CONSENSUS  EMBH44836anti GCP18-5CP418L-Sams GCP18-4CP418L-R2000 CONSE129bal-Dral GCPS18-129Sam-ba2 GCPS18-129Sam-ba2 GCPS18-129R20-ba2 GCPR18-3129R211-ba2 GCPR18-3129R211-ba2 GCPR18-5CP418L-Sams	CTGTAAA TG.TTCCAA CAAAA CCACT ACATAGCAGAGTTC CTG.AAAATAATTCCCTCAAAAATTAACATF TTC CTG.AAAAT.ATTCCCT CAAAA TTA. ACATT TTC CTG.AAAA.TAATTCCCT CAAAA TTA. ACAT. TTC CTG.AAA.TAATTCCCT CAAAA TTA. ACAT. TTC CTG.AAA.TAATTCCCT CAAAA TTA. ACAT. TTC  TACAA.A.  251  300 .CTATACC CTCCTATAAC TGTCTGT.AA ATGTCCAATC AAAACCACTA CAGAACAAAG TGTCTGT.AA ATGTCCAATC AAAACCACTA CAGAACAAAG TGTCTGT.AA ATGTCCAATC AAAACCACTA CAGAACAAAG TGTTTC
CONSE129bal-Drak GCPS18-129Sam-ba2 GCPR18-3129R211-ba2 GCP18-10129R20-ba2 CONSENSUS  EMBH44836anti GCP18-5CP418L-Sams GCP18-5CP418L-R2000 CONSE129bal-Dral GCPS18-129Sam-ba2 GCPS18-129Sam-ba2 GCPR18-3129R211-ba2 GCPR18-3129R211-ba2 GCPR18-10129R20-ba2 CONSENSUS	CTGTARA TG.TTCCAA CARAA CCACT ACATAGCAGAGTTC CTG ARAATAMTTCCCT CARAA TTA. ACATT TTC CTG ARAATAMTTCCCT CARAA TTA. ACATT TTC CTG ARAATAMTTCCCT CARAA TTA. ACATT TTC CTG ARAA.TAATTCCCT CARAA TTA. ACAT. TTC CTCTATACC CTCTATACC CTCCTATACC
CONSE129bal-Drak GCPS18-129Sam-ba2 GCPR18-3129R211-ba2 GCP18-10129R20-ba2 CONSENSUS  EMBH44836anti GCP18-5CP418L-Sams GCP18-4CP418L-R2000 conse129bal-Dral GCPS18-129Sam-ba2 GCPS18-129Sam-ba2 GCPS18-129R20-ba2 CONSENSUS  EMBH44836ant GCP18-5CP418L-Sams GCP18-2CP418L-Sams GCP18-2CP418L-Sams GCP18-2CP418L-Sams GCP18-2CP418L-Sams GCP18-2CP418L-Sams GCP18-2CP418L-R2000 conse129bal-Drag	CTGTARA TG.TTCCAA CARAR CCACT ACATAGCAGAGTTC CTG.AARATARTTCCCTCARARARTIAL. CATF TTC CTG.AARATARTCCCT CARAR TTAL. ACATT TTC CTG.AARATARTCCCT CARAR TTAL. ACATT TTC CTG.AARATARTCCCT CARAR TTAL. ACAT. TTC CTG.AARATARTCCCT CARAR TTAL. ACAT. TTC  T.ACAR.A.  251  300  CTATACC CTCCTATARC CTCCTATARC CTCCTATARC CTCCTATARC CTCTTATARC CTCCTATARC CTCCT
CONSE129bal-Drak GCPS18-129Sam-ba2 GCPR18-3129R211-ba2 GCP18-10129R20-ba2 CONSENSUS  EMBH44836anti GCP18-5CP418L-Sams GCP18-4CP418L-R2000 CONSE129bal-Dral GCPS18-129Sam-ba2 GCP18-3129R211-ba2 GCP18-10129R20-ba3 CONSENSUS  EMBH44836ant GCP18-5CP418L-Sams GCP18-2CP418L-Sams GCP18-2CP418L-R2000 CONSE129bal-Dral GCPS18-129Sam-ba3	CTGTARA TG.TTCCAA CARAA CCACT ACATAGCAGAGTTC CTG ARAATAMTTCCCT CARAR TTA. ACATT TTC TACAA.A.  CTG ARAATAMTTCCCT CARAR TTA. ACATT TTC T.ACAA.A.  CTG ARAA.TATTCCCT CARAR TTA. ACATT TTC T.ACAA.A.  CTG ARAA.TATTCCCT CARAR TTA. ACATT TTC T.ACAA.A.  251  300  CTATACC CTCTATACC CTCTATACC CTCCTATACC CTCC
CONSE129bal-Drak GCPS18-129Sam-ba2 GCPR18-3129R211-ba2 GCP18-10129R20-ba2 CONSENSUS  EMBH44836anti GCP18-5CP418L-Sams GCP18-4CP418L-R2000 CONSE129bal-Dral GCPS18-129Sam-ba2 GCP18-3129R211-ba2 GCP18-5CP418L-Sams GCP18-5CP418L-Sams GCP18-5CP418L-Sams GCP18-2CP418L-Sams GCP18-10129R20-ba2 CONSENSUS  EMBH44836ant GCP18-5CP418L-Sams GCP18-10129R20-ba2 CONSENSUS  EMBH44836ant GCP18-10129R20-ba2 CONSENSUS  EMBH44836ant GCP18-10129R20-ba2 GCP18-1129Sam-ba3 GCPS18-129Sam-ba3 GCPS18-129Sam-ba3 GCPR18-3129R211-ba3	CTGTARA TG.TTCCAA CARAA CCACT ACATAGCAGAGTTC CTG ARAATAATTCCCT CARAA TTA. ACATT TTC CTG ARAAT.ATTCCCT CARAA TTA. ACATT TTC CTG ARAA.TAATTCCCT CARAA TTA. ACATT TTC CTG ARAA.TAATTCCCT CARAA TTA. ACAT. TTC  300 .CTATACC CTCCTATARC CTCCTATAC CTCCTATAC CTCCTATAC CTCCTATAC CTCCTATAC CTCCTATAC CTCCTATAC CTC
CONSE129bal-Drak GCPS18-129Sam-ba2 GCPR18-3129R211-ba2 GCP18-10129R20-ba2 CONSENSUS  EMBH44836anti GCP18-5CP418L-Sams GCP18-4CP418L-R2000 CONSE129bal-Dral GCPS18-129Sam-ba2 GCP18-3129R211-ba2 GCP18-10129R20-ba3 CONSENSUS  EMBH44836ant GCP18-5CP418L-Sams GCP18-2CP418L-Sams GCP18-2CP418L-R2000 CONSE129bal-Dral GCPS18-129Sam-ba3	CTGTARA TG.TTCCAA CARAR CCACT ACATAGCAGAGTTC CTG ARARTANTTCCCT CARAR TTAL. ACATT TTC TACAR.A.  CTG ARARTANTTCCCT CARAR TTAL. ACATT TTC T.ACAR.A.  CTG ARARTANTTCCCT CARAR TTAL. ACATT TTC T.ACAR.A.  CTG ARARTANTTCCCT CARAR TTAL. ACATT TTC T.ACAR.A.  CTG ARARTANTTCCCT CARAR TTAL. ACATT. TTC T.ACAR.A.  SOO  CTATACC CTCTATACC CTCCTATARC CTC

# Figure 15 (b)

EMBH44836anti GCP18-5CP418L-Sams GCP18-2CP418L-Wes GCP18-4CP418L-R2000 conse129bal-Drak GCPS18-129Sam-ba2 GCPR18-3129R211-ba2 GCP18-10129R20-ba2 Consensus	400 TGAGCTTAAT ATCACCCAA. TGAGCTTAAT ATCACCCAAA TGAGCTTAAT ATCACCAAA TGAGCTTAAA TCTTATCTAAA G.TTATCAC ATCACAT GAA GA AAT CTTATCTAAA G.TTATCAC ATCACAT GAA GA
EMBH44836anti GCP18-5CP418L-Sams GCP18-2CP418L-Wes GCP18-4CP418L-R2000 conse129ba1-Drak GCPS18-129Sam-ba2 GCPR18-3129R211-ba2 GCP18-10129R20-ba2	ATCGTTTTGA GATTAGAACA AA
Consensus  EMBH44836anti GCP18-5CP418L-Sams GCP18-2CP418L-Wes GCP18-4CP418L-R2000 conse129ba1-Drak GCPS18-129Sam-ba2 GCPR18-3129R211-ba2 GCP18-10129R20-ba2 Consensus	431 CTGAAACTTA CGTAGAGTGA TTTGAGGAGT AGGCTCGTTG CCAGCAGAGC CTAAACCTTA CGTAGAGTGA TCTGAGGAGT AGGCTCGTTG CCAGCAGAGC CTAAACCTTA CCTAGAGTGA TCTGAGGAGT AGGCTCGTTG CCAGCAGAGC CTAAACTTA CCTAGAGTGA TCTGAGGAGT AGGCTCGTTG CCAGCAGAGC CTAAACTTA CCTAGAGTGA TCTGAGGAGT AGGCTCGTTG CCAGCAGAGC CTAAACTTA CCTAGAGTGA TCTGAGGAGT AGGCTCGTTG CCAGCAGAGC CTAAACCTTA CCTAGAGTGA TCTGAGGAGT AGGCTCGTTG CCAGCAGAGC CTAAACCTTA CCTAGAGTGA TCTGAGGAGT AGGCTCGTTG CCAGCAGAGC CTAAACCTTA CCTAGAGTGA TCTGAGGAGT AGGCTCGTTG CCAGCAGAGC CTAAACCTAGAGTGA TCTGAGGAGT AGGCTCGTTG CCAGCAGAGC CTAAACCTAGAGTGA TCTGAGGAGT AGGCTCGTTG CCAGCAGAGC CTAAACCTAGAGTGA TCTGAGGAGT AGGCTCGTTG CCAGCAGAGC CTAAACCTAGAGTGA TCTGAGGAGTA AGGCTCGTTG CCAGCAGAGC CTAAACCTAGAGTGA TCTGAGAGAGTA AGGCTCGTTG CCAGCAGAGC CTAAACTAGAGTGAGAGAGAGAGAGAGAGAGAGAGAG
EMBH44836anti GCP18-5CP418L-Sams GCP18-2CP418L-Wes GCP18-4CP418L-R2000 consel29bal-Drak GCPS18-129Sam-ba2 GCPR18-3129R211-ba2 GCP18-10129R20-ba2 Consensus	TAGCTCTCTC CTCCGCCTCA TGAAGCATCT GTTGCACCTG AGACAACCGT TAGCTCTCTC CTCCGCCTCA TGAAGCATCT GCTGCACCTG AGACAACCGT
EMBH44836anti GCP18-5CP418L-Sams GCP18-2CP418L-Wes GCP18-4CP418L-R2000 conse129ba1-Drak GCPS18-129Sam-ba2 GCPR18-3129R211-ba2 GCP18-10129R20-ba2 Consensus	531 GACGARACTT TCCGATCACC GCCACCAGAA TTCGACGCCG CGCATCGGAA TCCGATCACC GCCACCAGAA TTCGACGCCG CGCATCGGAA GACGARACTT TCCGATCCCC GCC.CCAGAA TTCGACGCCG CGCATCGGAA
EMBH44836ant: GCP18-5CP418L-Sam: GCP18-2CP418L-We: GCP18-4CP418L-R200: conse129ba1-Dra: GCPS18-129Sam-ba: GCPR18-3129R211-ba GCP18-10129R20-ba Consensu	GGATCCGAAT CGGGAACTGG AGTGAACCCG AGCGATCCCG GGAGTGCGAC GGATCCGAAT CGGGAACTGG AGTGAACCCG AGCGATCCCG GGAGTGCGAC GGATCCGAAT CGGGAACTGG AGTGAACCCG AGCGATCCCG GGAGTGCGAC GGATCCGAAT CGGGAACTGG AGTGAACCAG AGCGATCCCG GGAGTGCGAC GGATCCGAAT CGGCAACTGG AGTGAACCAG AGCGATCCCG GGAGTGCGAC GGATCCGAAT CGGCAACTGG AGTGAACCAG AGCGATCCCG GGAGTGCGAC GGATCCGAAT CGGGAACTGG AGTGAACCAG AGCGATCCCG GGAGTGCGAC

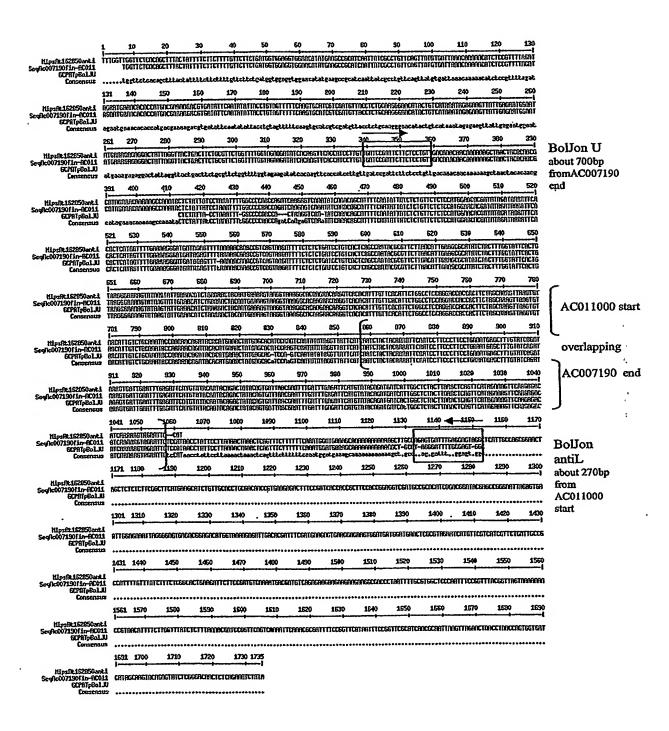
#### Figure 15 (c)

	631				690	
EMBH44836anti	GGAGCGATGG	GAAAAGAGAG	TGGCACGATT	TCGACGAAGA	GTGGAAGAGG	
GCP18-5CP418L-Sams	GGAGCGATGG	GAAAAGAGAG	TGGCACGATT	TCGACGAAGA	GTGGAAGAGG	
GCP18-2CP418L-Wes	GGAGCGATGG	GAAAAGAGAG	TGGCACGATT	TCGACGAAGA	GTGGAAGAGG	
GCP18-4CP418L-R2000	GGAGCGATGG	GAAAAGAGAG	TGGCACGATT	TCGACGAAGA	GTGGAAGAGG	
consel29bal-Drak	GGAGCGATGG	GAAAAGAGAG	TGGCACGATT	TCGACGAA.A	GTGGAAGAGG	
GCPS18-129Sam-ba2	GGAGCGTTGG	AAAAAGAGAG	TGGCACGATT	TCGACGAAGA	GAGGAAGAGG	
GCPR18-3129R211-ba2	GGAGCGTTGG	AAAAAGAGAG	TGGCACGATT	TCGACGAAGA	GAGGAAGAGG	
GCP18-10129R20-ba2					GAGGAAGAGG	
Consensus				tcgacgaaga		
00.100.10	99-9-9-100				• • • • • • • • • • • • • • • • • • • •	
	691				740	
EMBH44836anti				AAGTTCGTCA		
GCP18-5CP418L-Sams	AGAGGGTGGT	GGATAAACTC	GCGTATGATC	AAGTTCGTCA	TCGTCCTGAT	
GCP18-2CP418L-Wes	AGAGGGTGGT	GGATAAACTC	GCGTATGATC	AAGTTCGTCÄ	TCGTCCTGAT	
GCP18-4CP418L-R2000	AGAGGGTGGT	GGATAAACTC	GCGTATGATC	AAGTTCGTCA	TCGTCCTGAT	
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GCPS18-129Sam-ba2	AGAGGGTGGT	GGATAAACTC	GCGTATGATC	AAGTTCGTCA	TCGTCCTGAT	
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# Figure 16

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Converd - Caseling of American Converd - Converd Conve	172 A3200  170 COGGOCCETT  170 170 170 170 170 170 170 170 170 170	THE THAT THOSE THE THAT THOSE THE THAT THOSE THE THAT THOSE THAT THOSE THAT THAT THE THAT THAT THAT THAT THAT T	MACCITYI- TYCCCIATU MACCITYO- 1330 1330 1330 13400 14400 14400 14400 14400 14400 14400 14400 14400 14400	-THOTHSATCH YTTCTANATCH -THOGGSC -THOGGSC -THOGGSC -THOTHSTON HTTACTTON (TAMTIBOTON -TAMTIBOTON	TAGE	2/20 2/20 2/20 2/20 2/20 2/20 2/20 2/20	1600 1200 1200 1200 1200 1200	1514 1776805101 1776805101 1516117702 1516177702 1516177702 1516177702	TENTEN P. D.	ANNESCH PRINCIPES PO POCTUREN	ACCO  TYPE	TENTENGER TENTEN	TYTICCAST TYTICCAST TYTICCAST 1420 1420 1420 1420 1420 1420 1420 1420	1500 RETUTA THEATT INGTOTAL TH
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#### Figure 17



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